MODULE 2.6.1. INTRODUCTION

2.6.1. INTRODUCTION

There is an urgent need for the development of a new prophylactic vaccine given the threat posed by the increasing number of globally distributed outbreaks of Severe Acute Respiratory Syndrome (SARS)-CoV-2 infection and thus its associated disease Coronavirus Disease 2019 (COVID-19). A Lipid Nanoparticle (LNP)-formulated ribonucleic acid (RNA)-based vaccine would provide one of the most flexible, scalable, and fastest approaches to provide protection against new, fast spreading, virus infection. The development of an RNA-based vaccine encoding a viral antigen that is translated by the vaccinated organism to protein to induce a protective immune response provides significant advantages over more conventional vaccine approaches.

BNT162b2 (BioNTech code number BNT162, Pfizer code number PF-07302048) is a vaccine intended to prevent COVID-19, which is caused by SARS-CoV-2. BNT162b2, otherwise known as BNT162b2 (V9), is a nucleoside modified mRNA (modRNA) expressing full-length spike (S) protein with two proline mutations (P2) to lock the transmembrane protein in an antigenically optimal prefusion conformation. The vaccine is formulated in lipid nanoparticles. The LNP is composed of 4 lipids: ALC-0315, ALC-0159, DSPC, and cholesterol. Other excipients in the formulation include sucrose, NaCl, KCL, Na_2HPO_4, and KH_2PO_4. The drug product is a preservative-free, sterile dispersion of RNA formulated in LNP in aqueous cryoprotectant buffer for intramuscular (IM) administration. The RNA drug substance is the only active ingredient in the drug product. The drug product is a concentrate for injection and filled a (b) (4) mg/mL in glass vials and closed with stoppers and flip off crimping cap.

2.6.1.1. Proposed Indications

BNT162b2 is indicated for active immunization to prevent COVID-19 caused by SARS-CoV-2 virus, in individuals 16 years of age and older. The dose selected for BNT162b2 for commercial use is 30 ug RNA administered IM on Days 1 and 22.

MODULE 2.6.2 PHARMACOLOGY WRITTEN SUMMARY

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Abbreviation	Term
ACE2	angiotensin converting enzyme 2
BAL	Bronchoalveolar lavage
CDC	Centers for Disease Control
COVID-19	Coronavirus disease 2019
CT	Cytoplasmic tail
DART	Developmental and reproductive toxicology
dLIA	Direct Luminex immunoassay
DSPC	1,2-distearoyl-*sn*-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot
FITC	Fluorescein isothiocyanate
FP	Fusion peptide
GD	Gestation day
GFP	Green fluorescent protein
GMC	Geometric mean concentration
GMT	Geometric mean titer
HCS	Human convalescent sera
HR	Heptad repeat
HRP	Horseradish peroxidase
ICOS	inducible costimulatory molecule
ICS	Intracellular cytokine staining
IFA	immunofluorescence
IFN	Interferon
IgG	Immunoglobulin G
IL	interleukin
IM	intramuscular
IN	Intranasal
IT	intratracheal
kDa	kilodalton
LD	Lactation day
LLOQ	lower limit of quantification
mAb	Monoclonal antibody
MERS	Middle East respiratory syndrome
mL	milliliter
mNG	mNeongreen
modRNA	Modified mRNA
MW	molecular weight
NHP	Nonhuman primate
ORF	Open reading frame
P2 S	stable prefusion S including two proline substitutions
PBS	Phosphate-buffered saline
PD	Protease domain

Abbreviation	Term
PFU	Plaque forming unit
PND	Postnatal day
PVDF	Polyvinylidene fluoride
pVNT	Pseudotype neutralization titer
$pVNT_{50}$	50% pseudovirus neutralizing titer
$pVNT_{90}$	90% pseudovirus neutralizing titer
RNA	ribonucleic acid
RDRP	RNA-dependent RNA polymerase
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
S	SARS-CoV-2 spike glycoprotein
S1 / S2	SARS-CoV-2 spike glycoprotein subdomains 1 / 2
S2′	S2 protease cleavage site
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide electrophoresis
SEC	Size exclusion chromatography
SS	Signal sequence
Tfh	T follicular helper cell
TLR	Toll-like receptor
TM	transmembrane
TNF	tumor necrosis factor
µg	microgram
ULOQ	Upper limit of quantification
US	United States
USA	United States of America
VEE	Venezuelan equine encephalitis virus
VSV	Vesicular stomatitis virus
VNT_{50}	50% virus neutralizing titer
WHO	World Health Organization

2.6.2. PHARMACOLOGY WRITTEN SUMMARY

2.6.2.1. Introduction

BNT162b2 (BioNTech code number BNT162, Pfizer code number PF-07302048) is BNT162b2 (V9), a nucleoside-modified mRNA (modRNA) vaccine that encodes the SARS-CoV-2 full-length spike glycoprotein (S). In some preclinical research, a different variant of BNT162b2 was used: BNT162b2 (V8), which has a different codon optimization but encodes a protein with the same amino acid sequence as BNT162b2 (V9). In this document, "BNT162b2" refers to BNT162b2 (V9), unless otherwise specified. The glycoprotein encoded by both BNT162b2 variants includes two amino acid substitutions to proline (P2 S) locking the transmembrane protein in an antigenically optimal prefusion conformation (Pallesen et al, 2017; Wrapp et al, 2020).

The RNA is formulated with functional and structural lipids, which protect the RNA from degradation and enable transfection of the RNA into host cells after IM injection. The formulation contains two functional lipids, ALC-0315 and ALC-0159, and, two structural lipids, DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine) and cholesterol.

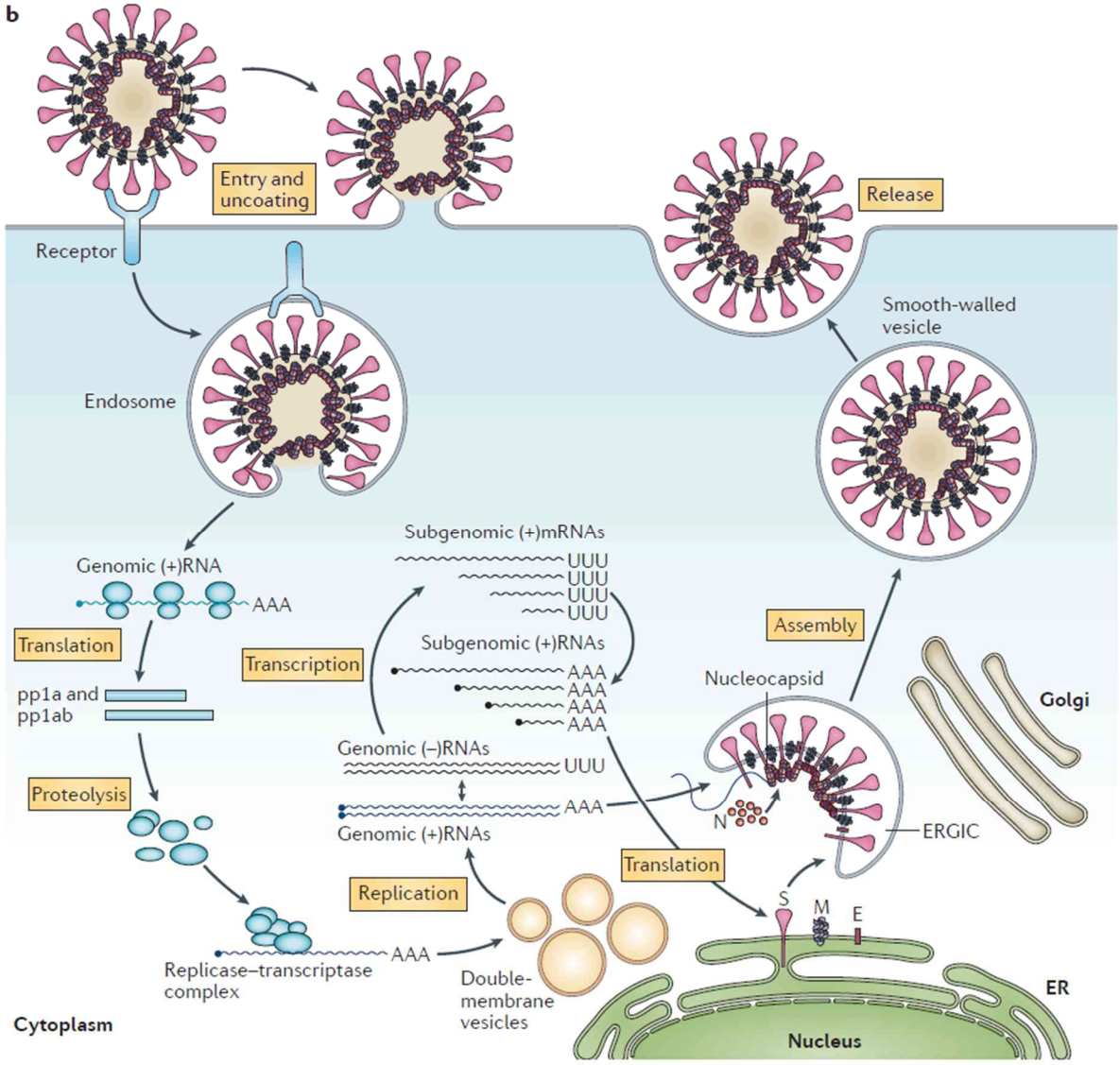
The modRNA comprises a single-stranded, 5′-capped mRNA that is translated upon entering the cell. In addition to the open reading frame (ORF) encoding the SARS-CoV-2 P2 S antigen, each modRNA contains common structural elements optimized for high translational efficacy of the RNA. ModRNA also contains a substitution of 1-methyl-pseudouridine for uridine. This substitution decreases recognition of the vaccine RNA by innate immune sensors, such as toll-like receptors (TLRs) 7 and 8, resulting in decreased innate immune activation and increased protein translation (Kariko et al, 2005). Vaccination with modRNA is characterized by the strong expansion of Th1-skewed antigen-specific T follicular helper (Tfh) cells, which stimulate and expand germinal center B cells, thereby resulting in particularly strong, long-lived, high-affinity antibody responses (Sahin et al, 2014; Pardi et al, 2018). The structural elements of BNT162b2 contain non-coding sequences optimized for prolonged and strong translation of the P2 S antigen-encoding RNA component.

2.6.2.2. SARS-CoV-2 S as a Vaccine Target

SARS-CoV-2 is an enveloped, positive sense, single-stranded RNA virus that is coated with S, which gives the virion its characteristic corona or "crown" appearance (Figure 2.6.2-1). Coronavirus S is a major target of virus neutralizing antibodies and is a key antigen for vaccine development. S is a transmembrane glycoprotein responsible for receptor recognition, attachment to the cell, and viral envelope fusion with a host cell membrane resulting in genome release, which is driven by the S conformation change leading to the fusion of viral and host cell membranes. For infection, S requires proteolytic cleavage by two host proteases, a furin-like protease between the S1 and S2 subunits, and by the serine protease TMPRSS2 at a conserved site directly preceding the fusion peptide (S2') (Figure 2.6.2-2; Bestle et al, 2020; Hoffmann et al, 2020). While the membrane-proximal S2 furin cleavage fragment is responsible for membrane fusion, the membrane-distal S1 fragment, with its receptor-binding domain (RBD), recognizes the host receptor and binds to the target host cell. SARS-CoV S and SARS-CoV-2 S have similar structural properties and

bind to the same host cell receptor, angiotensin converting enzyme 2 (ACE2) (Zhou et al,2020).

Figure 2.6.2-1. Replication Cycle of a Coronavirus

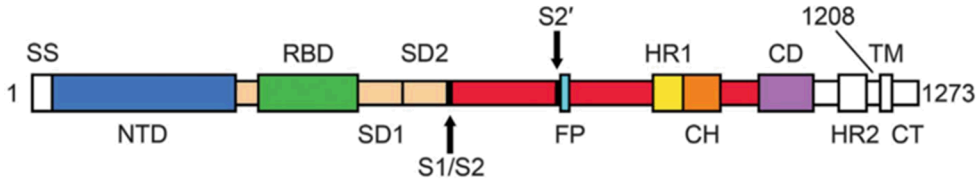


Source: de Wit et al, 2016

S is a large, trimeric glycoprotein that exists predominantly in a prefusion conformation on the virion (Ke et al, 2020). 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 critical for the induction of virus neutralizing antibodies by the host immune system (Zakhartchouk et al, 2007; Yong et al, 2019). Some monoclonal antibodies against S, particularly those directed against the RBD, neutralize SARS-CoV and Middle East respiratory syndrome (MERS)-CoV infection *in vitro* and *in vivo* (Hulswit et al, 2016). Vaccines targeting the S protein are sufficient to induce strong neutralizing immune responses (Al-Amri et al, 2017).

The RBD forms membrane distal "heads" on the S trimer that are connected to the body by a hinge. In the native S, the RBD alternates between an open (up) and closed (down) position. Although potent neutralizing epitopes have been described when the RBD is in the "heads down" closed conformation, the "heads up" receptor accessible conformation exposes a potentially greater breadth of neutralizing antibody targets (Brouwer et al, 2020; Liu et al, 2020; Robbiani et al, 2020). A P2 mutant (P2 S) variant of S contains two consecutive prolines introduced at amino acid positions 986 and 987, between the central helix (CH) and heptad repeat 1 (HR1) (Figure 2.6.2-2). These mutations lock S in the prefusion conformation (Pallesen et al, 2017; Wrapp et al, 2020). A proportion of P2 S has one RBD in the "heads up" and two RBDs in the "heads down" position, and there is probably a dynamic equilibrium as the heads hinge up and down (Cai et al, 2020; Henderson et al, 2020).

Figure 2.6.2-2. 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.

BNT162b2 (V9) encodes for a full-length P2 S. The V9 codon optimization variant contains a higher content of cytosine ribonucleotides than V8 for increased protein expression and is the focus of this marketing application. The RNA-expressed P2 S is membrane anchored. It elicits of a potent humoral neutralizing antibody response and Th1-type $CD4^+$ and $CD8^+$ cellular response to block virus infection and kill virus infected cells, respectively.

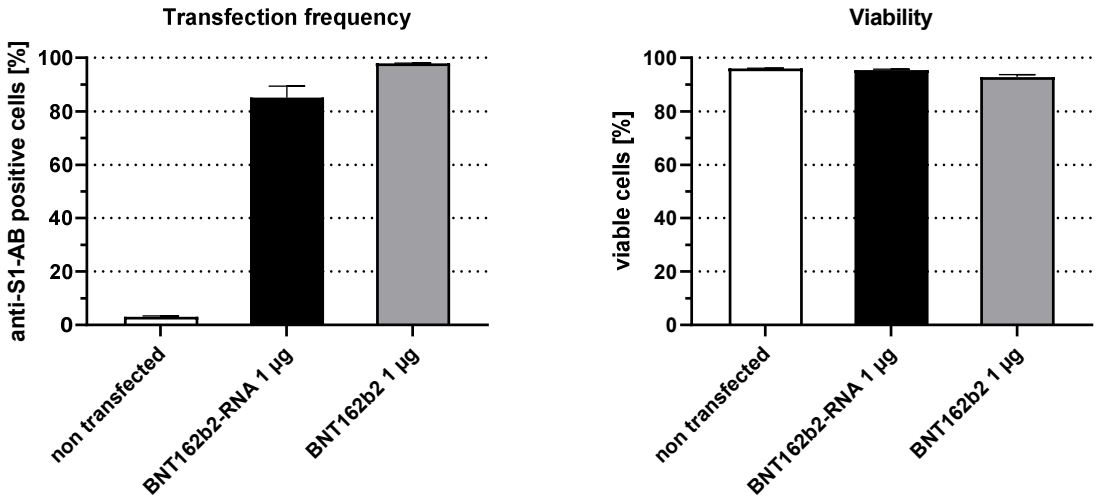
2.6.2.3. In Vitro Expression of Antigens from BNT162b2 (V9) RNA

Different in vitro methods were performed to analyze SARS-CoV-2 P2 S expression. To assess transfection frequencies in cells exposed to BNT162b2 RNA mixed with a commercial transfection reagent or exposed to BNT162b2 (which is LNP-formulated), flow cytometry analysis was performed. Immunofluorescence staining of transfected cells was used to assess cellular localization.

Flow cytometry analysis of HEK293T cells transfected with either BNT162b2 RNA or LNP-formulated BNT162b2 led to high frequencies of cells being transfected, with BNT162b2-transfected cells being transfected at a slightly higher frequency than cells exposed to BNT162b2 RNA mixed with a commercial transfection reagent (Figure 2.6.2-3).

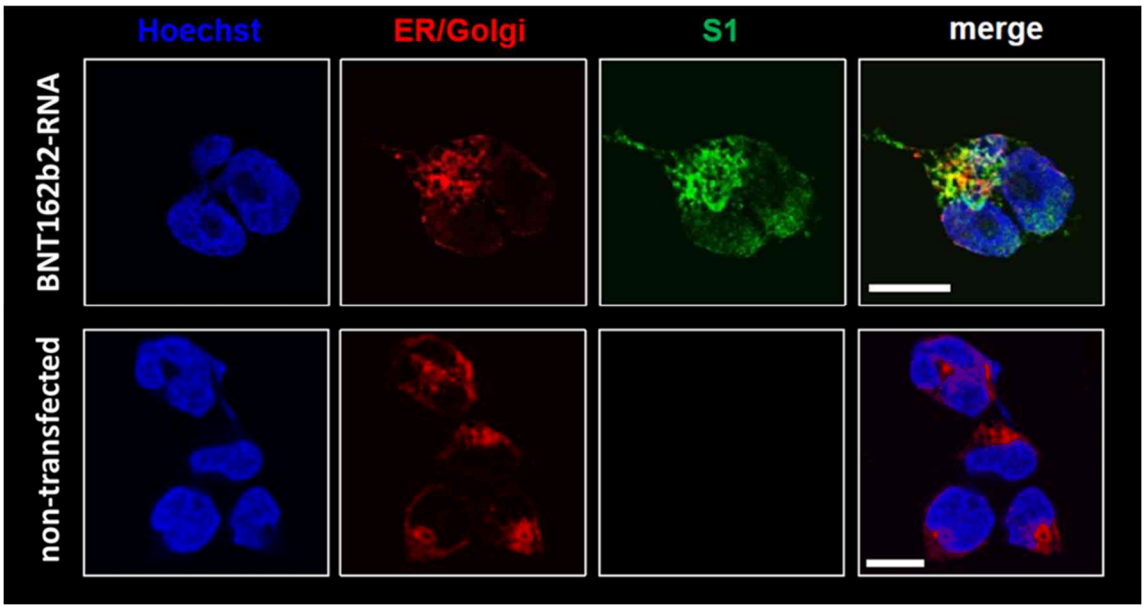
There were no differences in cell viability after transfection with BNT162b2 RNA or BNT162b2 compared to non-transfected cells. Furthermore, co-localization of the S protein antigen with an ER marker was detected by immunofluorescence experiments in HEK293T cells expressing BNT162b2-RNA suggesting the S protein is processed within the ER (Figure 2.6.2-4).

Figure 2.6.2-3. Flow Cytometry Analysis of BNT162b2 Transfection Frequency



HEK 293T cells were transfected using RiboJuice™ mRNA transfection reagent (Merck Millipore) with 1 µg of the RNA encoding BNT162b2 P2 S (V9) (BNT162b2 RNA) or the BNT162b2 (LNP-formulated RNA). After 18 h in culture, cells were stained with a viability dye, fixed, permeabilized and stained with a monoclonal rabbit antibody recognizing S1 and labelled with AlexaFluor647. Non-transfected cells were used as a control.

Figure 2.6.2-4. Immunofluorescence Detection of P2 S in BNT162b2 Transfected Cells

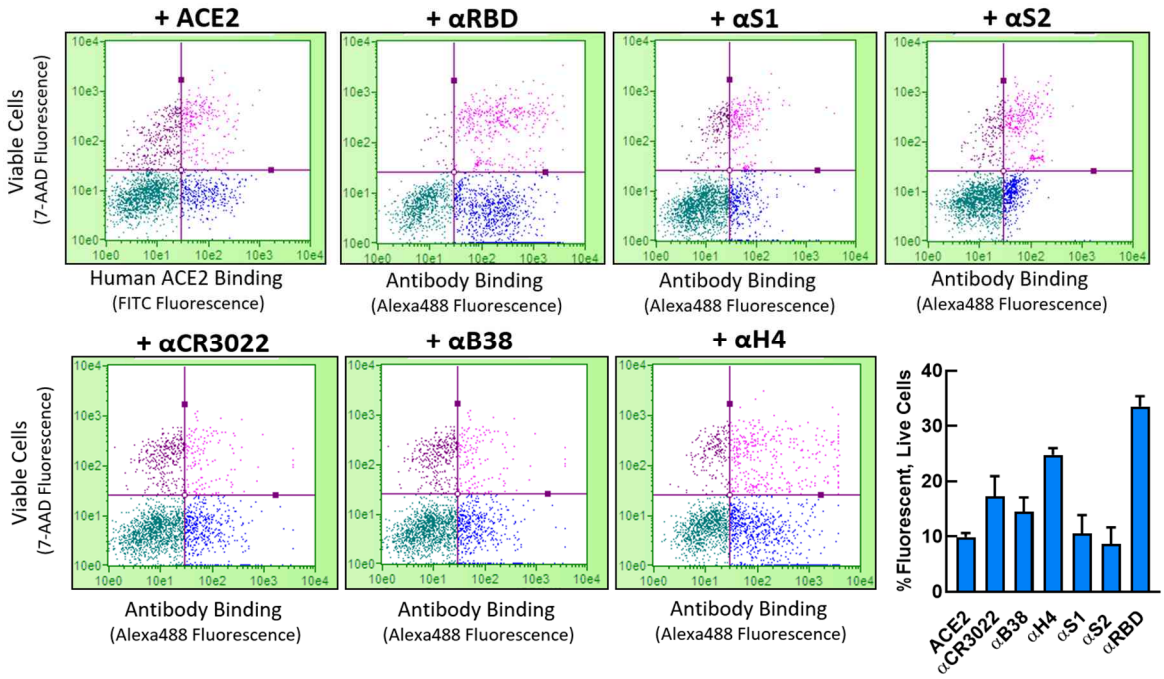


HEK293T cells were transfected with BNT162b2 (V9) RNA using RiboJuice™ RNA transfection reagent (Merck Millipore). After 18 h in culture, cells were fixed, permeabilized and stained for DNA to visualize the nucleus with Hoechst (blue), for the endoplasmic reticulum and Golgi (ER/ Golgi) with concanavalin A and Golgi tracker, both Alexa Fluor™ 594 conjugated (red). Cells were stained for P2 S with a monoclonal anti-S1 antibody and Alexa Fluor® 488 (green). The merged color panels show that the P2 S expressed by BNT162b2 (V9) colocalizes with the ER/ Golgi marker (scale: 10 µm). A control of non-transfected cells is shown in the lower row.

2.6.2.4. Structural and Biophysical Characterization of P2 S as a Vaccine Antigen

For structural characterization, P2 S was expressed in Expi293F cells from DNA that encodes the same amino acid sequence as BNT162b2 RNA, with the addition of a C-terminal TwinStrep tag for affinity purification (VR-VTR-10741). To confirm surface expression of untagged P2 S as well as the ability of P2 S to bind to human ACE2, flow cytometry experiments were performed on nonpermeabilized cells (Figure 2.6.2-5). Antibodies to the RBD, S1, and S2 were pre-incubated with Alexa-488 anti-IgG Fab for staining, and a nucleic acid dye was used to separate live and dead cells. To confirm binding of human ACE2, P2 S-expressing cells were labeled with the extracellular domain of human ACE2 pre-incubated with a FITC-labeled antibody against an affinity tag on the ACE2. Finally, anti-RBD human neutralizing antibodies B38 and H4 isolated from a COVID-19 convalescent patient (Wu et al, 2020) as well as the anti-RBD therapeutic antibody CR3022 (Yuan et al, 2020) were similarly confirmed to bind the surface-expressed P2 S.

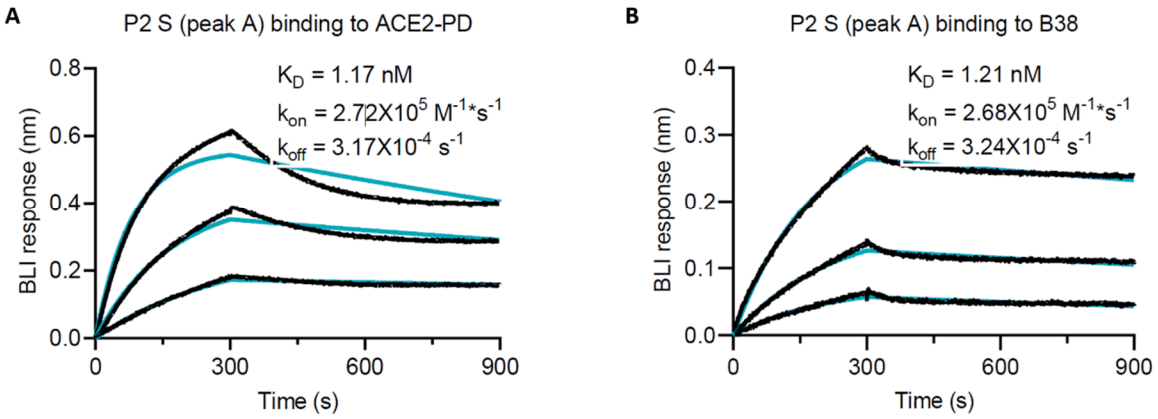
Figure 2.6.2-5. Binding to Cell Surface-Expressed Recombinant P2 S



P2 S antigen was over-expressed in Expi293F cells, and surface expression confirmed by staining with antibodies against the RBD, S1, and S2 regions of the full-length S protein. Human ACE2 extracellular domain (ACE2) as well as the therapeutic antibody CR3022 and two neutralizing antibodies isolated from a COVID-19 convalescent patient, B38 and H4, were further confirmed to bind to surface express P2 S. The nucleic acid dye 7-AAD was used identify viable cells (lower quadrants in flow plots). Binding to surface expressed P2 S over background in live cells is quantified across replicates in the bar graph.

Purification of the recombinant P2 S was based on a procedure described previously (Cai et al, 2020), with minor modifications. Upon cell lysis, P2 S was solubilized in 1% NP-40 detergent. The TwinStrep-tagged protein was then captured with StrepTactin Sepharose HP resin in 0.5% NP-40. P2 S was further purified by size-exclusion chromatography and eluted as three distinct peaks in 0.02 % NP-40 as previously reported (Cai et al, 2020). Protein from the first peak of a size exclusion column, containing intact P2 S and dissociated S1 and S2, was assayed by biolayer interferometry (Figure 2.6.2-6). The trimeric P2 S bound to the human ACE2 peptidase domain (ACE2-PD), and an anti-RBD human neutralizing antibody B38 with high affinity (apparent K_D = 1 nM).

Figure 2.6.2-6. Biolayer Interferometry Sensorgrams for Binding of P2 S to ACE2-PD and B38 mAb

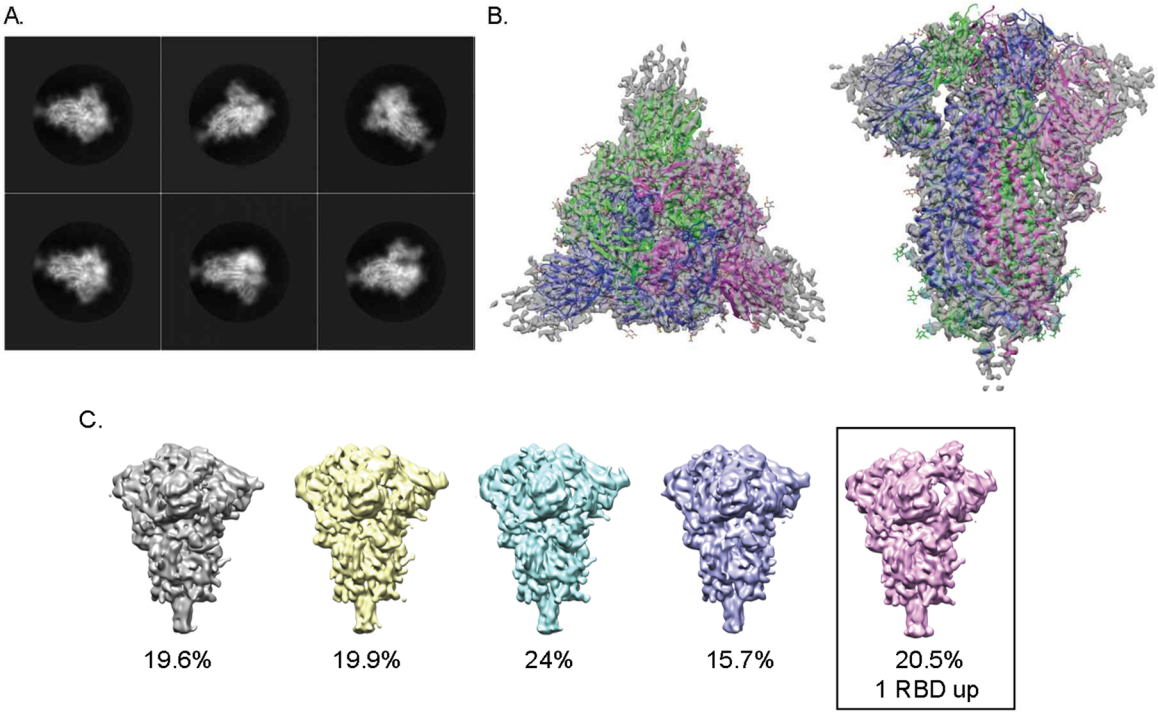


P2 S with a C-terminal TwinStrep tag expressed in Expi293F cells, was detergent solubilized and purified by affinity and size exclusion chromatography. Protein from the first peak of a size exclusion column, containing intact P2 S and dissociated S1 and S2, was assayed by biolayer interferometry on an Octet RED384 (FortéBio) at 25 °C in running buffer consisting of 25 mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA and 0.02 % NP-40. Sensorgrams showing the binding kinetics of TwinStrep-tagged P2 S to immobilized **A**, human ACE2-PD and **B**, B38 monoclonal antibody. The highest concentration tested for P2 S was 71 nM with 2 more 3-fold dilutions. The binding curves were globally fit to a 1:1 Langmuir binding model with R^2 values greater than 0.95. Actual binding data (black) and the best fit of the data to a 1:1 binding model (green). Apparent kinetic parameters are provided in the graphs.

Purified TwinStrep-tagged P2 S was characterized structurally using cryo-electron microscopy (cryoEM). 2D classification of particles from cryoEM data revealed a particle population that closely resembles the prefusion conformation of SARS-CoV-2 spike protein (Figure 2.6.2-7A). Processing and refinement of this dataset yielded a high-quality 3D map with a nominal resolution of 3.29 Å (Figure 2.6.2-7B), into which a previously published atomic model (PDB ID: 6VSB) was fitted and rebuilt. The rebuilt model shows good agreement with reported structures of prefusion full-length wild type S (Cai et al, 2020) and its ectodomain with P2 mutations (Wrapp et al, 2020). Three-dimensional classification of the dataset (Figure 2.6.2-7C) showed a class of particles that was in the one RBD ‘up’ (accessible for receptor binding), two RBD ‘down” (closed) conformation and represented 20.4% of the trimeric molecules. The remainder were in the all RBD ‘down’ conformation. The RBD in the ‘up’ conformation was less well resolved than other parts of the structure, suggesting conformational flexibility and a dynamic equilibrium between RBD ‘up’ and RBD ‘down’ states as also suggested by others (Cai et al, 2020; Henderson et al, 2020).

The well-resolved trimeric prefusion structure and the high affinity binding to ACE2 and human neutralizing antibodies demonstrate that the recombinant P2 S authentically presents the ACE2 binding site and other epitopes targeted by many SARS-CoV-2 neutralizing antibodies.

Figure 2.6.2-7. CryoEM P2 S Structure at 3.29 Å Resolution



A. 2D class averages of TwinStrep-tagged P2 S particles extracted from cryoEM micrographs. Box size is 39.2 nm in each dimension. **B**. 3.29 Å cryoEM map of TwinStrep-tagged P2 S, with fitted atomic model, showing top (perpendicular to the three-fold axis) and side (parallel to the three-fold axis) views. CryoEM model is based on PDB 6VSB and was fitted into the structure using manual rebuilding in Coot and real-space refinement in Phenix. ~28,000 micrographs were collected using a Titan Krios electron microscope operating at 300 kV accelerating voltage, and image processing and 3D reconstructions were performed using Warp and RELION. **C**. Maps of P2 S produced by 3D classification indicate some heterogeneity in positioning of the RBD domains. Percentages of the particle population represented in each class are indicated below the models.

2.6.2.5. Immunogenicity of BNT162b2 (V9) in Mice

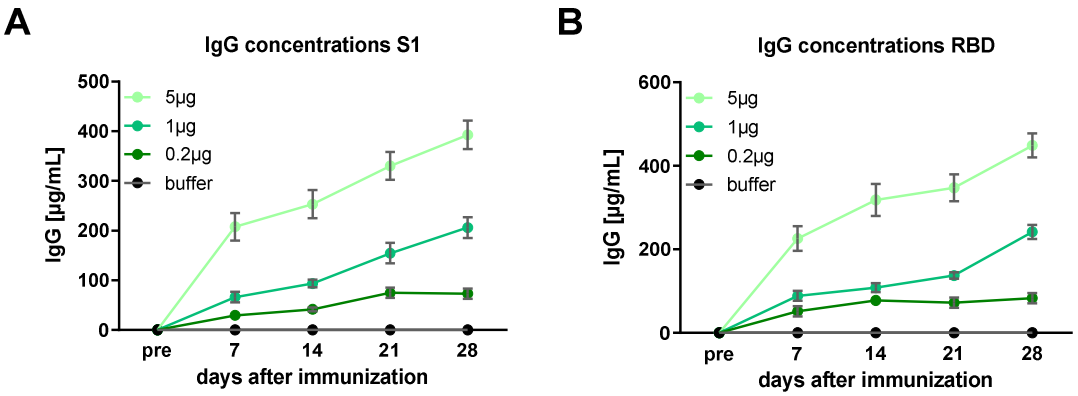
The immunogenicity of BNT162b2 (V9) in mice was investigated (Report R-20-0085).

Four groups of eight female Balb/c mice were immunized on day 0 with 0.2 µg, 1 µg or 5 µg RNA/animal of BNT162b2, or with buffer alone (control group). Blood was collected on Days 7, 14, 21 and 28 after immunization to analyze the antibody response by SARS-CoV2- RBD or S1 IgG ELISA and pseudotype neutralization (pVNT) (detailed methods described in Section 2.6.2.12.1 for ELISA and Section 2.6.2.12.2 for pVNT). Binding kinetics of SARS-CoV-2 S1- and RBD-specific IgGs were determined with sera generated at Day 28.

Immunization with BNT162b2 induced IgGs that bind S1 and RBD, while these antibodies were not detected in samples from buffer control animals. A dose-dependent increase in S1-binding IgGs was observed. Antibody concentrations in the serum samples were calculated using a mouse IgG monoclonal standard, and the kinetics of IgGs against S1 and

RBD are shown in Figure 2.6.2-8. At Day 28, the differences in concentrations of IgGs against S1 and RBD in the test groups compared to the buffer control group were statistically significant (S1: $p = 0.0259$ for 0.2 µg, $p < 0.0001$ for 1 µg and 5 µg; RBD: $p = 0.0072$ for 0.2 µg, $p < 0.0001$ for 1 µg and 5 µg) .

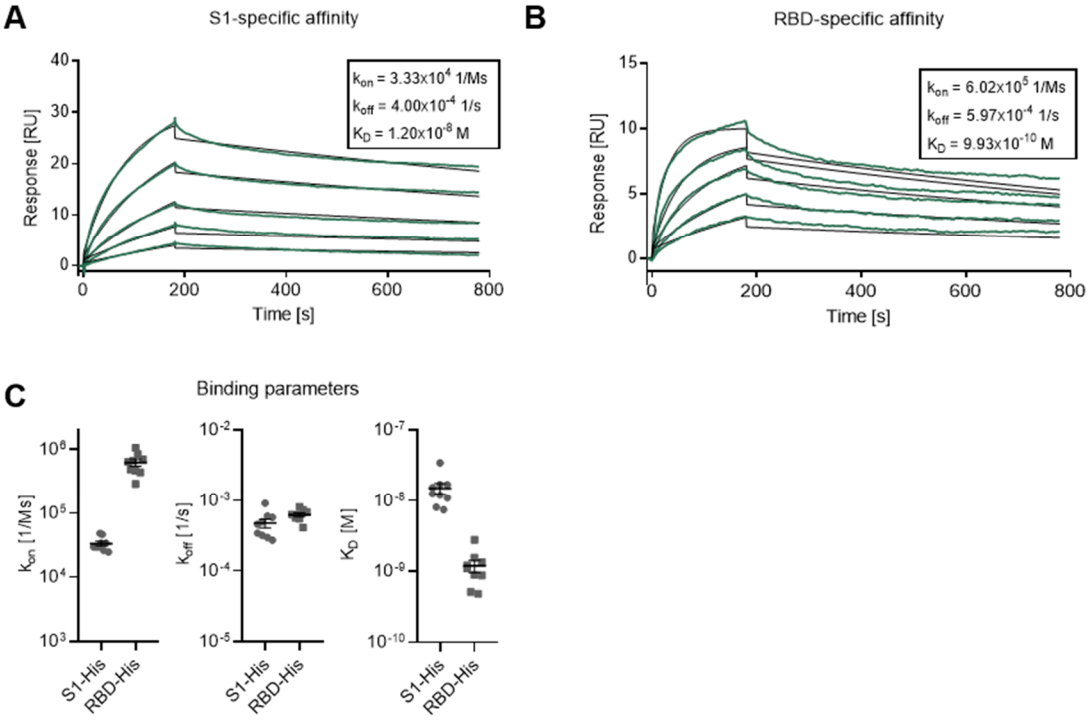
Figure 2.6.2-8. Anti-S IgG Response 7, 14, 21, and 28 d after Immunization with BNT162b2



BALB/c mice were immunized IM once with 0.2, 1 and 5 µg BNT162b2 or buffer. On 7, 14, 21, and 28 days after immunization, animals were bled. For individual ΔOD values, the antibody concentrations in the serum samples were calculated. The serum samples were tested by ELISA against (A) recombinant S1 and (B) recombinant RBD. Group mean antibody concentrations are shown (±SEM). Group size n=8. Statistical significance of the differences in IgG concentrations between the test groups and the control group was assessed by one-way ANOVA test with Dunnett's multiple comparison post-test on day 28.

At Day 28 after immunization, vaccine-elicited IgG against the S1 domain showed a very strong binding affinity (geometric mean KD 12 nM) including IgG binding the RBD with high affinity (geometric mean KD 0.99 nM), both with high on-rate (geometric mean kon: 3.33 x 104/Ms for S1-specific affinity; 6.02 x 105/Ms for RBD-specific affinity) and low off-rate (geometric mean koff: 4.00 x 10-4/s for S1-specific affinity; 5.97 x 10-4/s for RBD-specific affinity) (Figure 2.6.2-9).

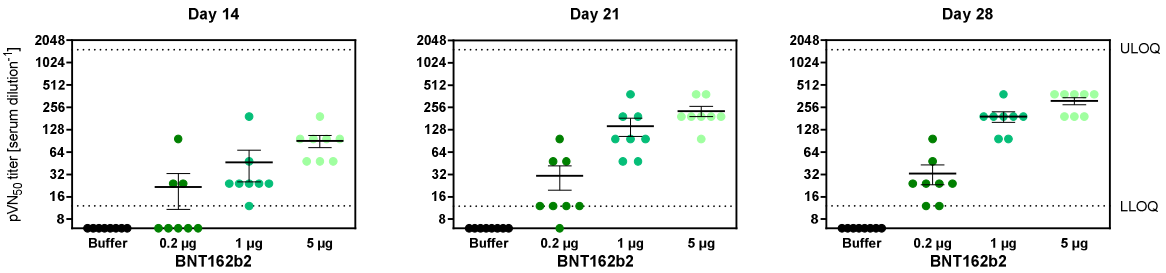
Figure 2.6.2-9. Binding Kinetics of Murine SARS-CoV-2 S1- and RBD-specific IgGs



BALB/c mice were immunized IM once with 5 µg BNT162b2. On Day 28 after immunization, animals were bled. IgG in the sera were tested for binding to recombinant histidine-tagged S1 (A) or recombinant histidine tagged RBD (B) (Sino Biological) using surface plasmon resonance spectroscopy in multi-cycle mode with concentrations ranging from 25-400 nM (S1-His) or 1.562-50 nM (RBD-His). Binding kinetics were calculated using a global kinetic fit to a 1:1 Langmuir model. Binding parameters are given in (C). Actual binding data (black) and the best fit of the data to a 1:1 binding model (green). One point in the graphs stands for one mouse. Group size n=8. Mean ± SEM is shown by horizontal bars with whiskers for each group.

In pVNT analysis, dose-dependent increases in neutralizing antibodies were observed (Figure 2.6.2-10).

Figure 2.6.2-10. BNT162b2 Pseudovirus Neutralizing Titers 14, 21, and 28 d after Immunization



BALB/c mice were immunized IM once with 0.2, 1 and 5 µg BNT162b2 or buffer. On 14, 21, and 28 d after immunization, animals were bled. The sera were tested for SARS-CoV-2 pseudovirus neutralization. Graphs depict $pVNT_{50}$ serum dilutions (50% reduction of infectious events, compared to positive controls without serum). One point in the graphs stands for one mouse. Every mouse sample was measured in duplicate. Group size n=8. Mean ± SEM is shown by horizontal bars with whiskers for each group. LLOQ, lower limit of quantification. ULOQ, upper limit of quantification.

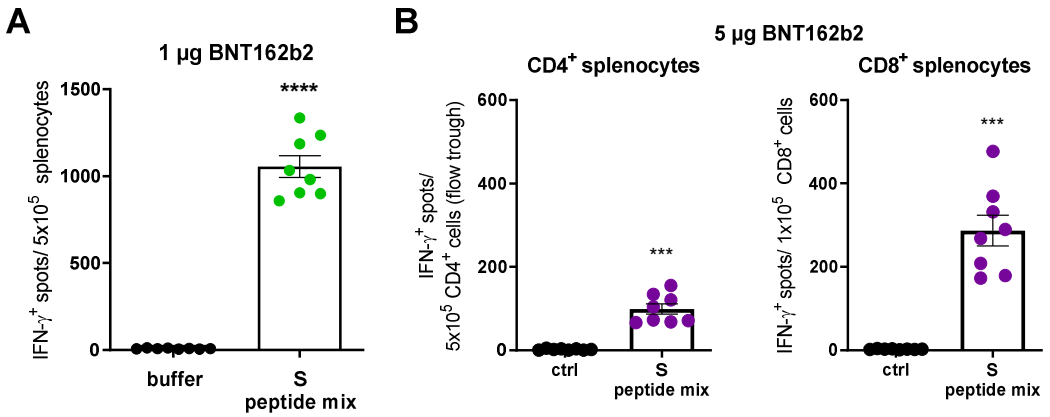
The summary of antibody titers on Day 28 is as follows:

Table 2.6.2-1. Summary of IgG Concentrations at Day 28 Post Immunization

	BNT162b2 0.2 µg	BNT162b2 1 µg	BNT162b2 5 µg
Anti S1 total IgG [µg/mL]	73.0 ± 10.4	205.9 ± 21.0	392.7 ± 28.9
Anti RBD total IgG [µg/mL]	83.1 ± 12.3	241.7 ± 17.2	448.6 ± 28.6
pVN_{50} titer [reciprocal dilution]	33.0 ± 9.8	192.0 ± 31.4	312.0 ±35.1

In addition, the cellular immune response was analyzed. At Day 28 after one immunization, mice were sacrificed and splenocytes were isolated to test for IFNγ release after antigen stimulation by ELISpot. Stimulation of fresh splenocytes with an S-specific overlapping peptide pool induced IFNγ responses in T cells of immunized animals. Splenocytes of the groups immunized with BNT162b2 had significantly higher spot numbers than splenocytes from the groups that received buffer control (Figure 2.6.2-11). To identify the T-cell subtype, an additional ELISpot analysis was performed after separation of fresh $CD4^+$ and $CD8^+$ cells by MACS isolation from splenocytes obtained from the group immunized with 5 µg BNT162b2. Both $CD4^+$ and $CD8^+$ cells displayed IFNγ responses.

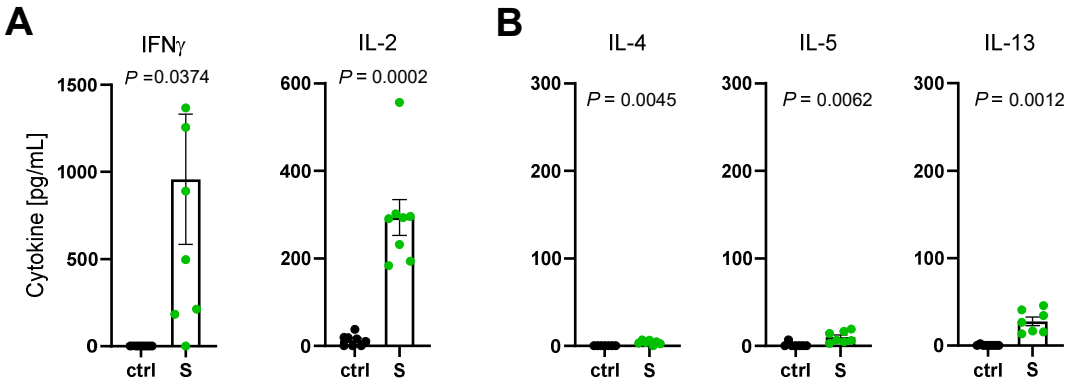
Figure 2.6.2-11. ELISpot Analysis Using Splenocytes Obtained on Day 28 after One Immunization



ELISpot assay was performed using (A) bulk splenocytes isolated on Day 28 after IM immunization of mice with 1 µg BNT162b2 or (B) CD4+ and CD8+ splenocytes after magnetic cell separation from the 5 µg BNT162b2 immunized group. Splenocytes were stimulated with S-specific overlapping peptide pools, buffer or an irrelevant control peptide (ctrl), and IFN-γ secretion was measured to assess S-specific T-cell number. Individual spot counts are shown by dots; group mean values are indicated by bars (±SEM). P-values were determined by one-way ANOVA analysis followed by Dunnett's multiple comparisons test. *** $p < 0.001$, **** $p < 0.0001$.

Furthermore, cytokine release data from the S-peptide mix stimulated splenocytes was acquired 28 days after immunization with 5 µg BNT162b2. High levels of the Th1 cytokines IFNγ and IL-2 but minute amounts of the Th2 cytokines IL-4, IL-5 and IL-13 in multiplex immunoassays were detected (Figure 2.6.2-12).

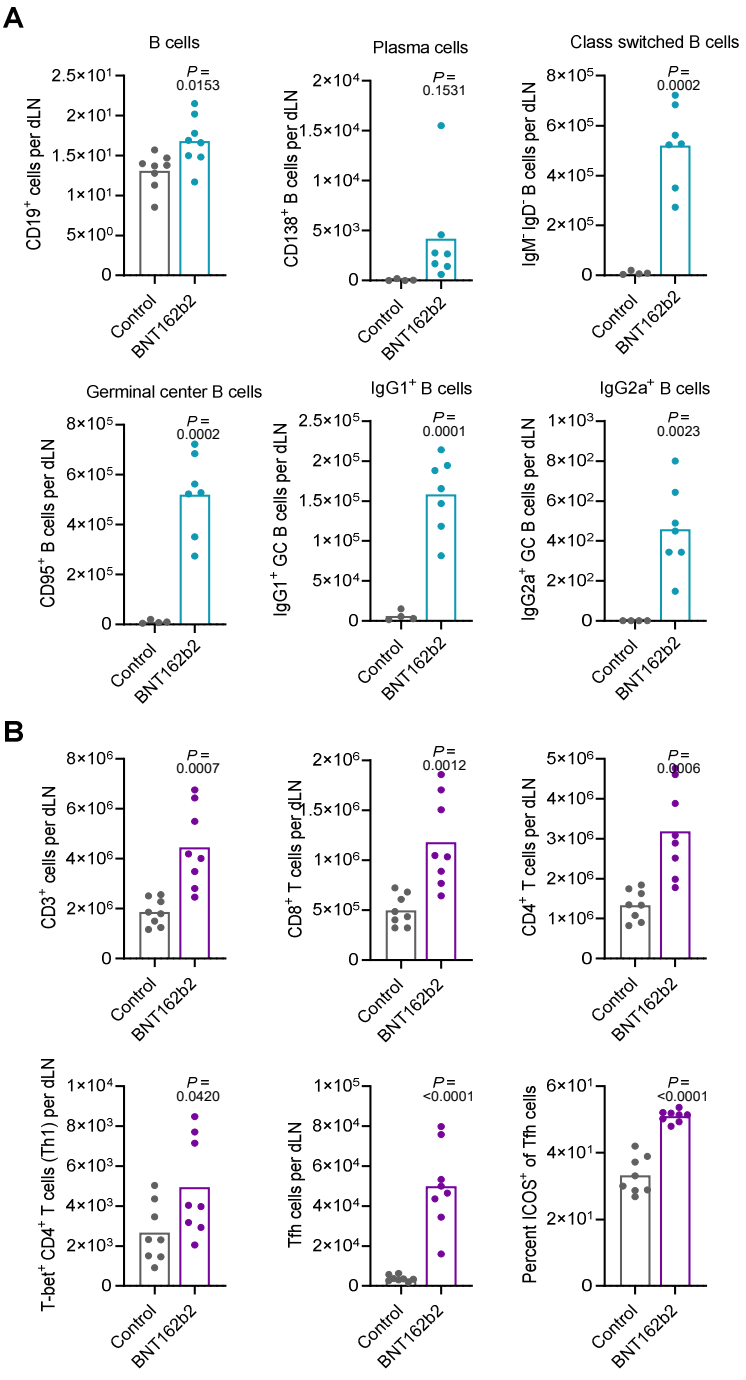
Figure 2.6.2-12. Cytokine Release Analysis Using Splenocytes Obtained on Day 28 after One Immunization



Splenocytes of BALB/c mice immunized IM with 1 µg BNT162b2 were stimulated *ex vivo* with full-length S peptide mix and cytokine multiplex analysis of supernatants was performed (n=8 per group). Splenocytes of buffer treated mice served as control. Cytokine production was determined by bead-based multiplex analysis (n=8 per group, n=7 for IL-4, IL-5 and IL-13 as one outlier was removed via routs test [Q=1%] for the S peptide stimulated samples). Individual dots indicate results from one animal; group mean values are indicated by bars. P-values were determined by a two-tailed paired t-test.

To dissect the cellular response after BNT162b2 immunization in more detail, mice were immunized with 5 µg BNT162b2 and 12 days after immunization draining lymph nodes (dLNs) were collected to perform B-cell and T-cell phenotyping analysis by flow cytometry (Figure 2.6.2-13). Much higher numbers of B cells (including plasma cells, class switched IgG1- and IgG2a-positive B cells, and germinal center B cells) were observed in the samples from mice that received BNT162b2 compared to controls. In addition, dLNs from BNT162b2-immunized mice also displayed an elevation in T-cell counts, particularly numbers of T follicular helper (Tfh) cells, including subsets with ICOS upregulation, which is known to play an essential role in the formation of germinal centers (Hutloff 2015).

Figure 2.6.2-13. B- and T-cell Phenotyping in Lymph Nodes of BNT162b2 Immunized Mice



Mice (n=8 per group) were immunized with 5 µg BNT162b2 or buffer (Control). (A) B-cell and (B) T-cell numbers 12 days after immunization in the subsets indicated by the y-axis labels were analysed in draining lymph nodes by flow cytometry. P-values were determined by an unpaired two-tailed t-test. The percentage of ICOS$^+$ cells among T follicular helper cells (Tfh) in draining lymph nodes (dLNs) is depicted on the lower right.

In summary, BNT162b2 (V9) induced a strong antibody response, with high total IgG, high binding affinity to S1 and the RBD, and high pVNT titers. Both $CD4^+$ and $CD8^+$ T-cell responses were detectable 12 and 28 days after one immunization with an overall significant increase in T-cell reactivity compared to control animals. Taking the phenotyping of B and T cells in aggregate, the data indicate a strong and concurrent induction of SARS-CoV-2 S-specific neutralizing antibody titers and a Th1-driven T-cell response by BNT162b2.

2.6.2.6. BNT162b2 (V9) Vaccine Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques

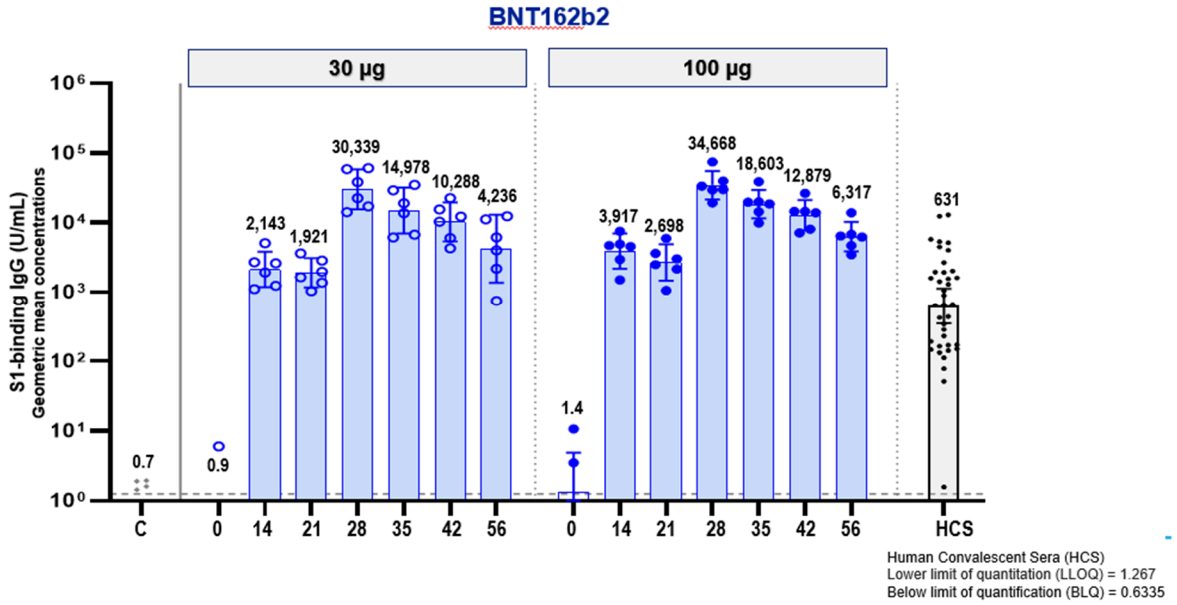
The ability of BNT162b2 immunization to protect rhesus macaques from live SARS-CoV-2 challenge was evaluated in 2–4 year old male rhesus macaques (VR-VTR-10671).

2.6.2.6.1. Immunogenicity in Rhesus Macaques

Groups of 2-4 year old male 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 after a single immunization, and levels increased further seven days after the second immunization (Day 28) to geometric mean S1-binding IgG concentrations (GMCs) of 30,339 units (U)/mL (30 µg dose level) and 34,668 U/mL (100 µg dose level) (Figure 2.6.2-14). For comparison, the GMC of a panel of 38 SARS-CoV-2 convalescent human sera was 631 U/mL, substantially lower than the GMC of the immunized rhesus macaques after one or two doses.

Human convalescent sera (HCS) were drawn from SARS-CoV-2 infected individuals 18 to 83 years of age, at least 14 days after PCR-confirmed diagnosis, and at a time when individuals were asymptomatic. The serum donors predominantly had symptomatic infections (35/38), and one had been hospitalized. Based on the assumptions that the immune response to SARS-CoV-2 infection provides some measure of protection from disease upon subsequent exposure to the virus and that the neutralizing antibody response contributes to that protection, the neutralizing antibody titer of the convalescent serum panel provides a currently assessable benchmark to judge the quality of the immune response to the vaccine candidates.

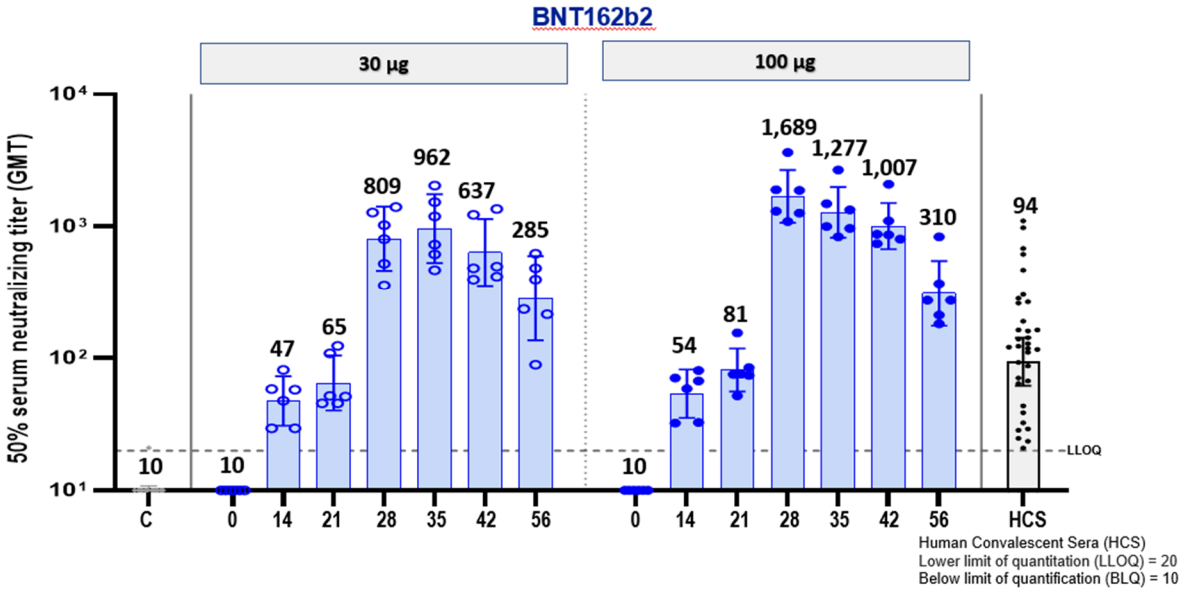
Figure 2.6.2-14. S1-Binding IgG Levels Elicited by Immunization of Rhesus Macaques with BNT162b2



S1-binding IgG concentrations elicited by immunization of rhesus macaques with BNT162b2. Numbers on the x-axis indicate the day post first immunization. Heights of bars indicate geometric mean concentrations (GMCs) in arbitrary units, 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 1.151 U/ml). Values at or below LLOQ were set to ½ LLOQ. C – saline-immunization control; HCS – human convalescent serum panel.

Fifty percent neutralization titers (VNT_{50}), measured by an authentic SARS-CoV-2 neutralization assay (Muruato et al, 2020), were detectable in rhesus sera by Day 14 after a single immunization and peaked at geometric mean titers (GMTs) of 962 (on Day 35, 14 days after Dose 2 of 30 µg) or 1,689 (on Day 28, 7 days after Dose 2 of 100 µg; Figure 2.6.2-15). Robust neutralization GMTs of 285 for 30 µg and 310 for 100 µg dose levels persisted to at least Day 56 (most recent time point tested). For comparison, the 50% neutralization GMT of the human convalescent serum panel was 94.

Figure 2.6.2-15. 50% Serum Neutralizing Titers Elicited by Immunization of Rhesus Macaques with BNT162b2

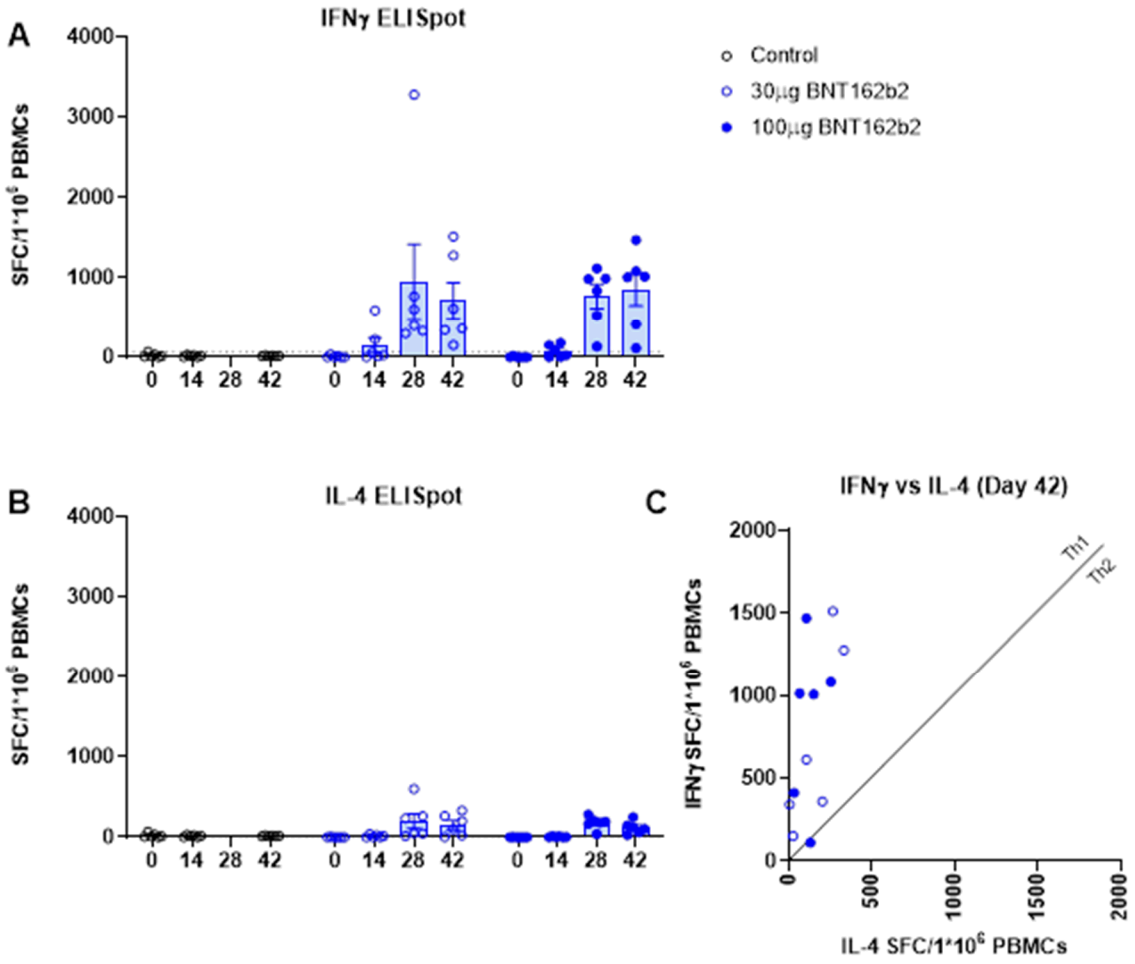


Numbers on the x-axis indicate the day post first immunization. Heights of bars indicate GMTs, which are written above the bars; whiskers indicate 95% confidence intervals; dots represent individual monkey titers. LLOQ – 20. Titers at or below LLOQ were set to ½ LLOQ. C – saline-immunization control; HCS – human convalescent serum panel.

Antigen-specific T-cell responses play an important role in generation of antigen-specific antibody response as well as in elimination of infected cells to mediate protection against disease. S-specific T-cell responses were analyzed in animals immunized with 30 µg or 100 µg of BNT162b2 and unimmunized controls (Control) by ELISpot and intracellular cytokine staining (ICS). PBMCs were collected before immunization (day 0), 14 days post dose 1 (14d PD1), 7 days post dose 2 (7d PD2), and 21 days post Dose 2 (21d PD2).

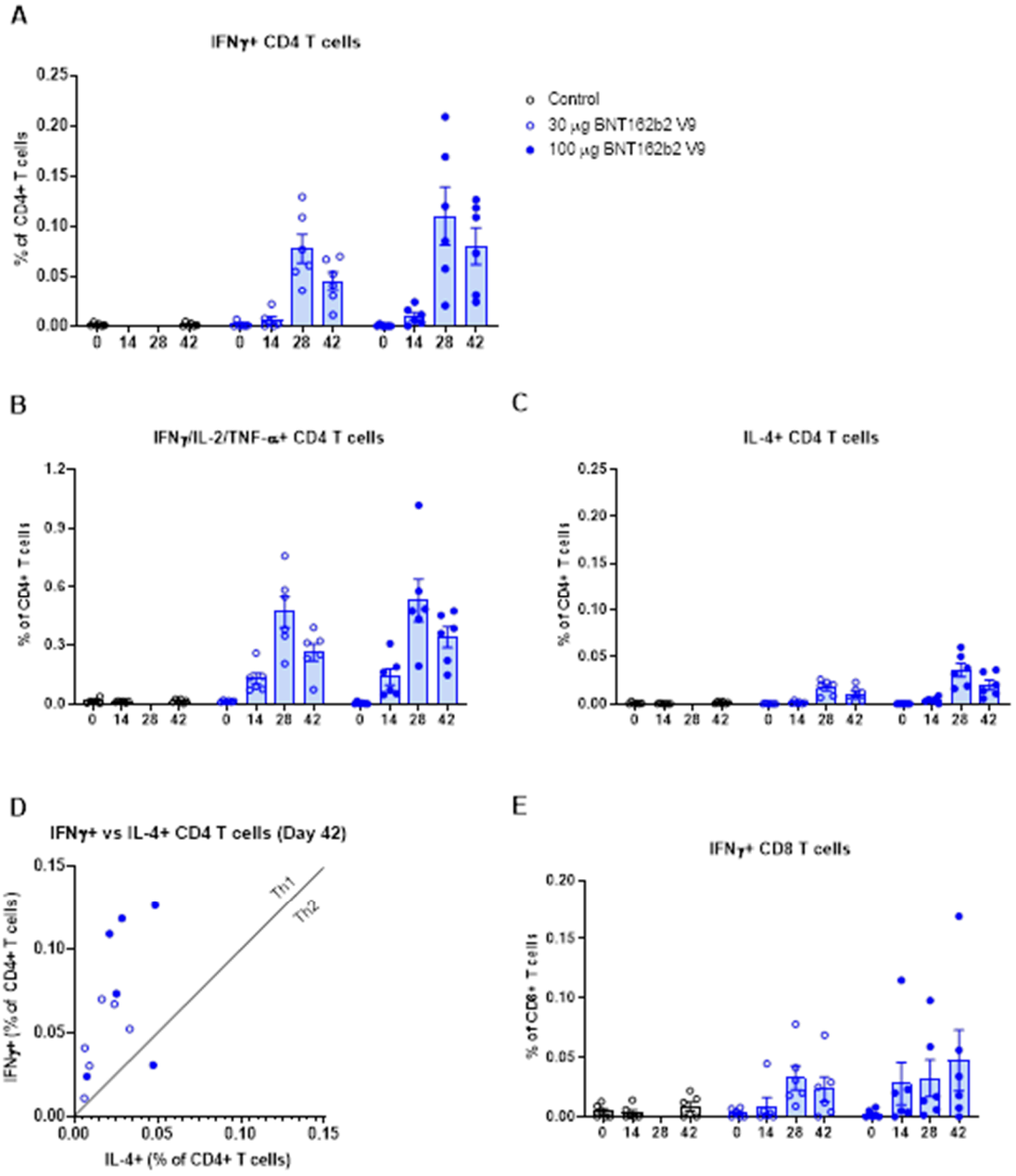
S-specific T cells were low to undetectable in naïve animals. Strong IFNγ ELISpot responses but minimal IL-4 ELISpot responses were detected after the second 30 or 100 µg dose of the vaccine candidate (Figure 2.6.2-16). ICS confirmed that BNT162b2 elicited strong S-specific IFNγ producing T cell responses, including a high frequency of $CD4^+$ T cells that produced IFNγ, IL-2, or TNF-α but a low frequency of $CD4^+$ cells that produce IL-4, indicating a Th1-biased response (Figure 2.6.2-17A through D). BNT162b2 also elicited S-specific IFNγ producing $CD8^+$ T cell responses (Figure 2.6.2-17E).

Figure 2.6.2-16. IFNγ and IL-4 ELISpot Results in BNT162b2 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 buffer (Placebo). Height of bars indicates the mean; whiskers indicate the standard error of mean (SEM); and each symbol represents one animal. Dotted lines mark the lower limit of detection. (A) IFNγ (B) IL-4 ELISpot analysis. (C) Correlation of frequency of IFNγ or IL-4 producing cells 21 days PD2.

Figure 2.6.2-17. S-specific CD4 and CD8 T-cell Response in BNT162b2 Immunized Animals as Measured by ICS Assay



Height of bars indicates the mean; whiskers indicate the standard error of mean (SEM); and each 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 or IL-4$^+$ CD4 T cells at 21 days PD2. (E) Frequency of IFNγ$^+$ CD8 T cells.

2.6.2.6.2. SARS-CoV-2 Challenge of BNT162b2 (V9)-Immunized Nonhuman Primates

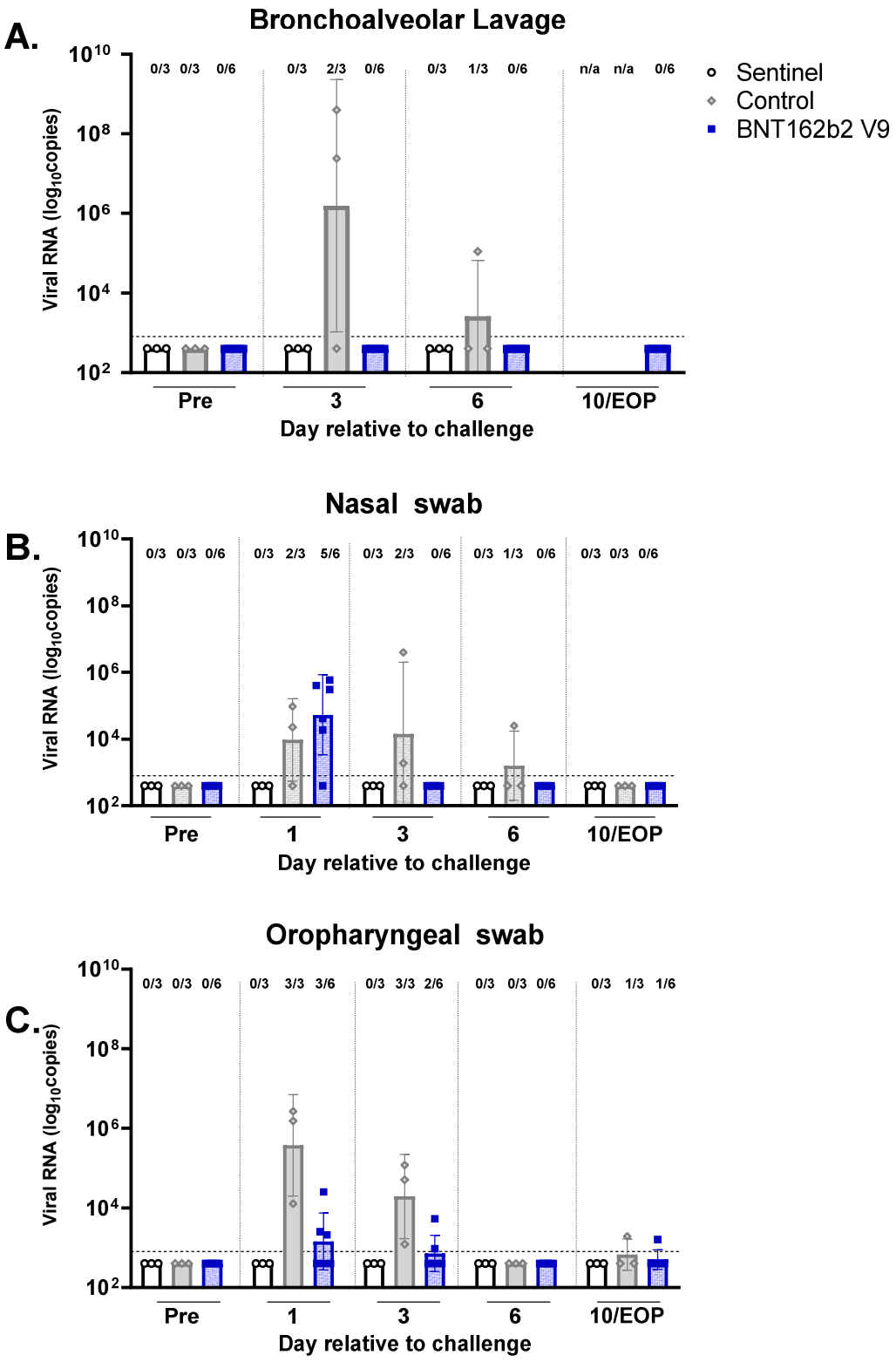
SARS-CoV-2 infection and COVID-19 in humans present diverse manifestation of signs, symptoms, and severity. Based on published reports, SARS-CoV-2 challenged rhesus macaques develop an acute, transient infection in the upper and lower respiratory tract and have evidence of viral replication in the gastrointestinal tract, similar to humans (Zou et al, 2020; Kim et al, 2020). Varying degrees of pulmonary inflammation, primarily at the peak of infection at approximately day 2 to 4 post-challenge, have been reported in the literature (Munster et al, 2020). The human and rhesus ACE2 receptor have 100% amino acid identity at the critical binding residues, which may account for the fidelity of this SARS-CoV-2 animal model (Zhou et al, 2020).

The groups of 2-4 year old male rhesus macaques that had received two intramuscular immunizations with 100 µg BNT162b2 (V9) (n=6) or buffer (Control; n=3) 21 days apart (described in Section 2.6.2.6.1) were challenged 55 days after the second immunization with 1.05×10^6 plaque forming units of SARS-CoV-2 (strain USA-WA1/2020), split equally between the intranasal and intratracheal routes, as previously described (Singh et al, 2020). Three additional non-immunized, age-matched, rhesus macaques (sentinel) were mock-challenged with cell culture medium. Nasal and oropharyngeal (OP) swabs were collected and bronchoalveolar lavage (BAL) was performed at the times indicated, and the samples were tested for the presence of SARS-CoV-2 RNA (genomic RNA and subgenomic transcripts) by reverse-transcription quantitative polymerase chain reaction (RT-qPCR; Figure 2.6.2-18). All personnel performing the clinical, radiographic, histopathologic, and RT-qPCR evaluations were blinded to the group assignments of the macaques (VR-VTR-10671).

All samples obtained before the infectious challenge and all those obtained from sentinel animals lacked detectable SARS-CoV-2 RNA (Figure 2.6.2-18). Viral RNA was detected in BAL fluid from 2 of the 3 control-immunized macaques on Day 3 after challenge and from 1 of 3 on Day 6. At no time point sampled was viral RNA detected in BAL fluid from the BNT162b2 (V9)-immunized and SARS-CoV-2 challenged macaques (Figure 2.6.2-18A). The difference in viral RNA detection in BAL fluid between BNT162b2-immunized and control-immunized rhesus macaques after challenge is statistically significant (p=0.0014).

From control-immunized macaques, viral RNA was detected in nasal swabs obtained on Days 1, 3, and 6 after SARS-CoV-2 challenge; from BNT162b2 (V9)-immunized macaques, viral RNA was detected only in nasal swabs obtained on Day 1 after challenge and not in swabs obtained on Day 3 or subsequently (Figure 2.6.2-18B). The pattern of viral RNA detection from OP swabs was similar to that for nasal swabs (Figure 2.6.2-18C).

Figure 2.6.2-18. Viral RNA in BAL Fluid and Nasal and Oropharyngeal Swabs of Rhesus Macaques after Infectious SARS-CoV-2 Challenge

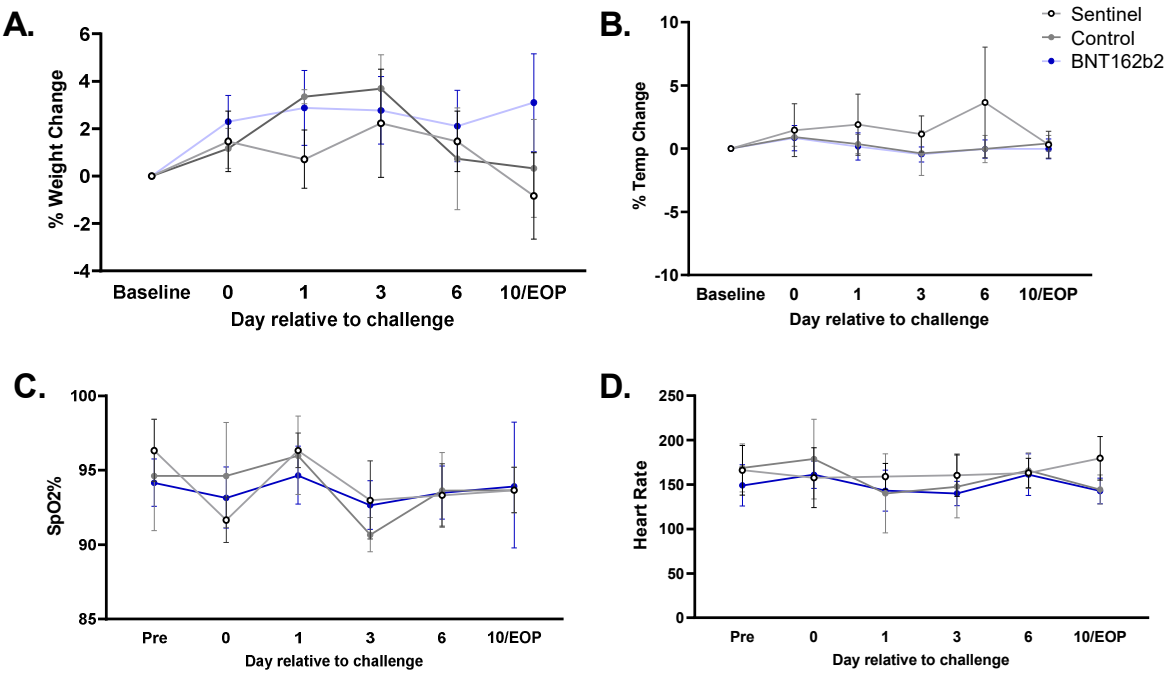


Groups of 2-4 year old rhesus macaques were immunized on days 0 and 21 with 100 µg BNT162b2 (V9) (n=6), or buffer (Control; n=3). Fifty-five days after the second immunization, the animals were challenged with 1.05×10^6 pfu of SARS-CoV-2 split equally between the IN and IT routes. Three age-matched male rhesus macaques were unimmunized and challenged with cell culture medium only (Sentinel). Viral RNA levels were detected by RT-qPCR in A) bronchoalveolar lavage, B) nasal swabs, and C) oropharyngeal swabs. EOP, end of project. 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.

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 2.6.2-19), indicating that the 2-4 year old male rhesus monkey challenge model appears to be an infection model, but not a clinical disease model. Lung radiograph (Figure 2.6.2-20A) and computerized tomography (CT) (Figure 2.6.2-20B) scores were determined by two board-certified veterinary radiologist who were blinded to treatment group. Data in Figure 2.6.2-20 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. 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 (Figure 2.6.2-21).

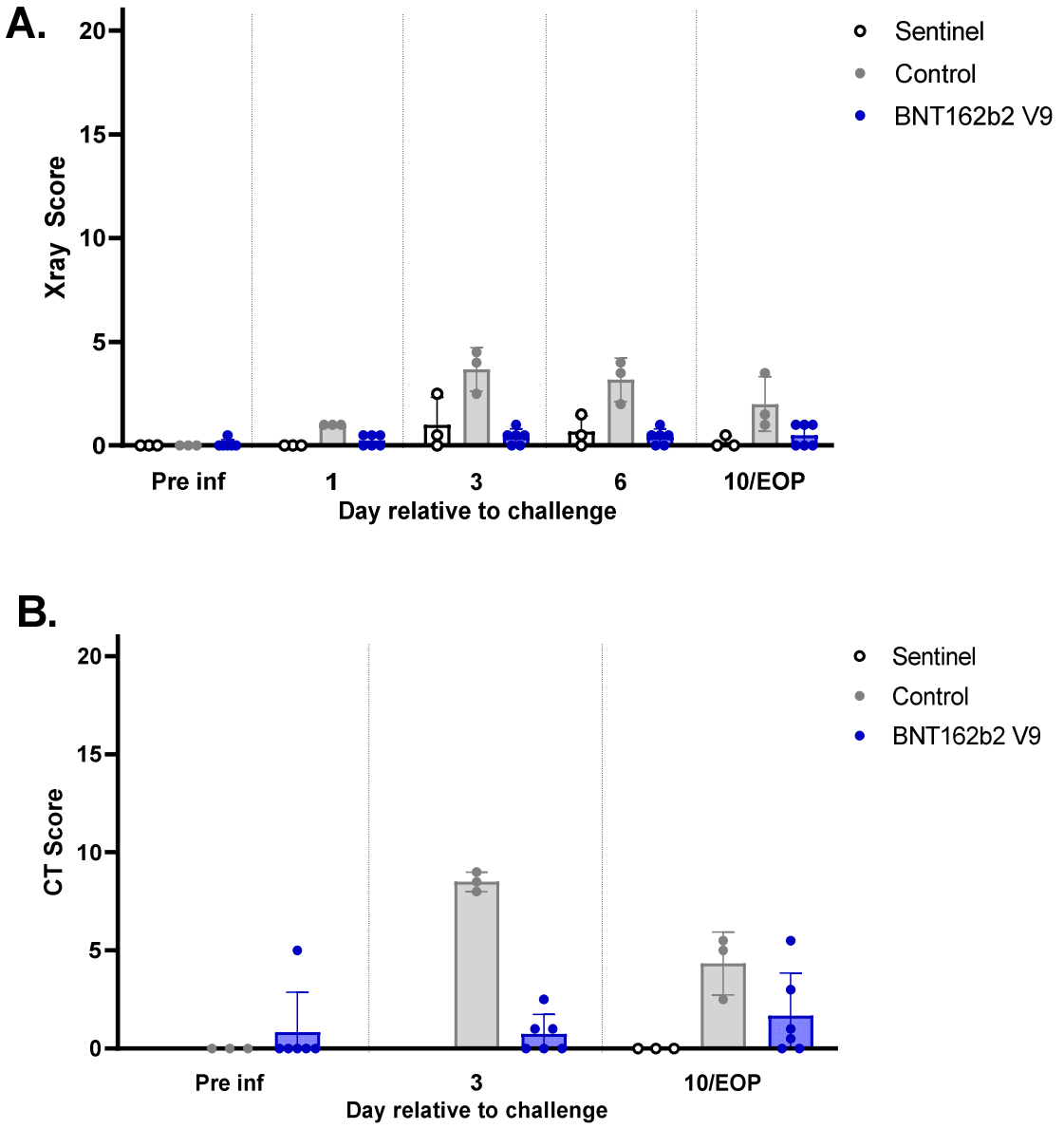
Overall, these data demonstrate that, compared to control, BNT162b2 (V9) immunization provided complete protection in the lungs from infectious SARS-CoV-2 challenge in rhesus macaques with no evidence of vaccine-elicited disease enhancement.

Figure 2.6.2-19. Clinical Signs in Rhesus Macaques after Immunization with BNT162b2 and Challenge with Infectious SARS-CoV-2



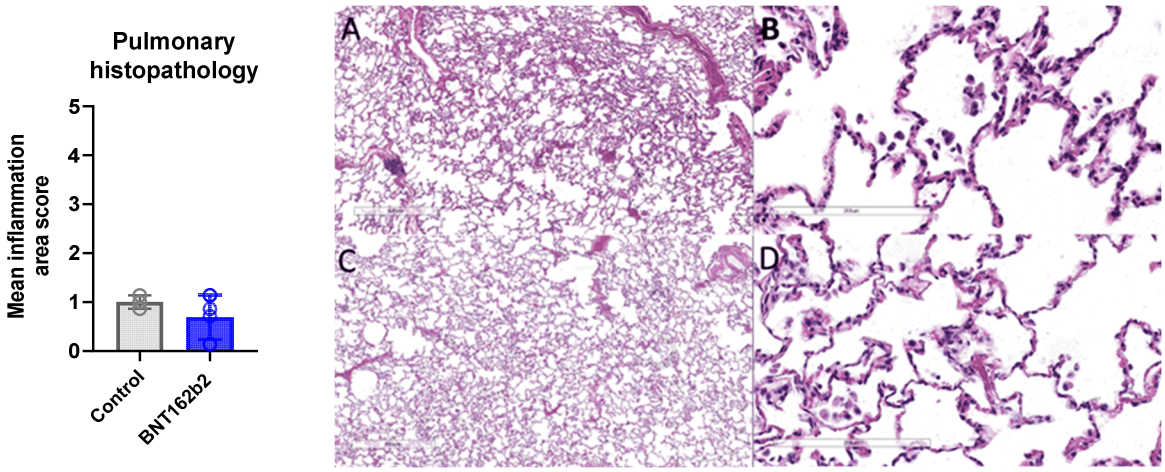
Rhesus macaques were immunized with BNT162b2 (V9), or saline, and challenged with SARS-CoV-2 or cell culture medium as described in the Figure 2.6.2-18 legend. Clinical signs were recorded on the days indicated. EOP, end of project. BNT162b2-immunized (n=6), control (n=3), and sentinel (n=3) macaques. A, Body weight. B, Temperature. C, Oxygen saturation. D, Heart rate.

Figure 2.6.2-20. 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.

Figure 2.6.2-21. 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).

2.6.2.7. Immunogenicity Testing of Rats in the GLP Compliant Repeat Dose Toxicity Studies and Developmental and Reproductive Toxicity Study

Immunogenicity results from two GLP-compliant repeat-dose toxicity studies, one (Study 20GR142) with BNT162b2 (V9) and one (Study 38166) with its closely related variant BNT162b2 (V8), as well as a DART study (Study 20256434) with BNT162b2 (V9) are presented below.

2.6.2.7.1. Repeat-Dose Toxicity Study of Three LNP-Formulated RNA Platforms Encoding for Viral Proteins by Repeated Intramuscular Administration to Wistar Han Rats

The immunogenicity of BNT162b2 (V8) in the GLP--compliant repeat-dose rat toxicity study (Study 38166) was analyzed. BNT162b2 (V8) has an alternative coding sequence to the Phase 2/3 study clinical candidate and subject of this application, BNT162b2 (V9), with V9 containing a higher content of cytosine ribonucleotides for increased protein expression. Both variants, V8 and V9, encode the identical protein, and in this toxicology study, the V8 was a surrogate for BNT162b2 (V9).

Male and female Wistar Han rats received three weekly doses of 100 µg of BNT162b2 (V8). Serum samples were collected and analyzed (5 animals/sex) from main study animals on Day 17, two days after the 3rd administration, at the end of the dosing phase as well as from recovery cohorts at the end of the study on Day 38. Treatment with the BNT162b2 vaccine elicited binding IgG against the S1 fragment and the RBD of SARS-CoV2 S. There was a

strong antibody response at both analyzed time points. The group mean IgG concentration against S1 and RBD are given in Table 2.6.2-2. Antibody concentrations against S1 and RBD increased over time.

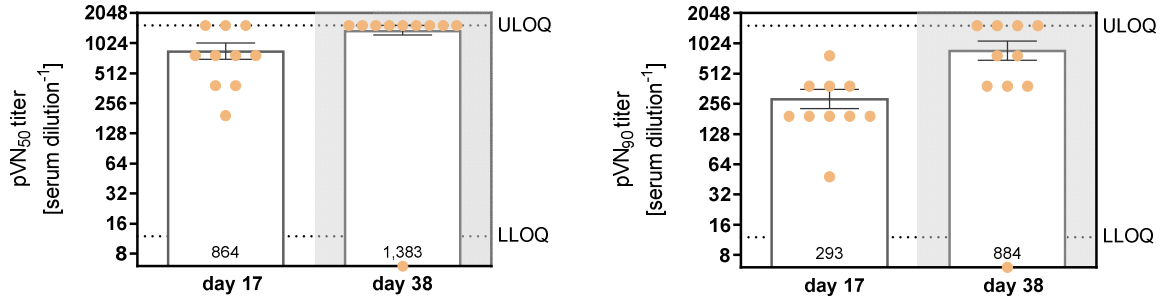
Table 2.6.2-2. IgG antibody Concentration [mg/mL] Against the Viral Antigen in Wistar Han Rats after BNT162b2 (V8) Immunization

		BNT162b2 (100 µg)
17 days after first immunization	**Against S1**	1.76 ± 0.16
	Against RBD	2.33 ± 0.19
38 days after first immunization	**Against S1**	3.46 ± 0.52
	Against RBD	4.90 ± 0.87

Pseudovirus neutralization results mirrored the antigen binding results.

Treatment of rats with BNT162b2 (V8) resulted in the elicitation of neutralizing antibodies against pseudovirus infection. Neutralizing antibody titers in vaccinated animals increased over time with the recorded neutralizing activity being consistent with the ELISA data shown above. Serum titers resulting in 50% pseudovirus neutralization exceeded the upper limit of quantification (ULOQ) of a reciprocal titer of 1536 in more than 8 out of 10 animals on Day 38, and therefore a neutralization titer of 90% was evaluated as well (Figure 2.6.2-22).

Figure 2.6.2-22. Pseudovirus Neutralization Activity in Rats after BNT162b2 V8 Immunization



Wistar Han rats were immunized IM with three weekly injections of 100 µg BNT162b2. On Day 17 and Day 38, animals were bled, and the sera were tested for titers of pseudovirus neutralizing antibodies. Individual titers resulting in 50% pseudovirus neutralization ($pVNT_{50}$, left graph) or 90% pseudovirus neutralization ($pVNT_{90}$, right graph) are shown by dots; group mean values are indicated by horizontal bars and are included in the figure (±SEM, standard error of the mean). Group size for analysis was n=5 male and n=5 female rats. Mean titers are given in the bars. All control serum samples were below the lower limit of quantification (LLOQ); ULOQ = upper limit of quantification.

2.6.2.7.2. 17-Day Intramuscular Toxicity Study of BNT162b2 (V9) in Wistar Han Rats With a 3-Week Recovery

The immunogenicity of the COVID-19 vaccine candidate BNT162b2 (V9) (and BNT162b3c) in the GLP compliant repeat-dose rat toxicity study (Study 20GR142) was analyzed. The summary of the results described below will focus on only the BNT162b2 (V9) candidate.

Wistar Han rats (15/sex/group) were administered IM doses of 0 (saline) or 30 BNT162b2 (V9) µg RNA/dose per animal. Doses were administered once a week for 3 weeks (Days 1, 8, and 15). Following the dosing phase, 10 animals/sex from each group were euthanized 2 days post last immunization for post-mortem assessments. The remaining 5 animals/sex/group were euthanized following a 3-week recovery phase.

Administration of 3 once-weekly doses of BNT162b2 (V9) elicited SARS-CoV-2 neutralizing antibody responses in males and females at the end of the dosing (Day 17) and recovery (Day 21) phases of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals (Table 2.6.2-3).

Table 2.6.2-3. Group Mean Titers of SARS-CoV-2 Neutralizing Antibodies

Study Day	Sex	Saline (0 µg RNA)	BNT162b2 (V9) (30 µg RNA)
Prior to Dosing Initiation (Day -5)	Male	5	5
	Female	5	5
End of Dosing Phase (Day 17)	Male	5	1114
	Female	5	2501
End of Recovery Phase (RP Day 21)	Male	5	5120
	Female	5	5120

RP = Recovery phase.

2.6.2.7.3. A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by Intramuscular Administration in the Wistar Han Rat

The immunogenicity of the COVID-19 vaccine candidate BNT162b2 (V9) (and BNT162b3c) in the GLP compliant DART (Study 20256434) was analyzed. The summary of the results described below will focus on only the BNT162b2 (V9) candidate.

Female Wistar Han rats (44 animals/group) were administered saline or 30 µg RNA/dosing day of BNT162b2 (V9) by IM injection for a total of 4 doses (21 and 14 days prior to mating and on GDs 9 and 20). On GD 21, half of the females in each group underwent Caesarean section. The remaining females in each group were allowed to naturally deliver their pups and both maternal animals and their offspring were monitored out through the end of weaning (LD 21/PND 21). SARS-CoV-2 neutralizing antibodies were assessed in maternal animals prior to mating, on GD 21, and LD 21 as well as in fetuses on GD 21 and in pups on PND 21.

BNT162b2 elicited SARS-CoV-2 neutralizing antibody responses in all of the females just prior to mating (M 0), at the end of gestation (GD 21), and at the end of lactation (LD 21). SARS-CoV-2 neutralizing titers were detected in all offspring (fetuses on GD 21 and pups on PND 21). SARS-CoV-2 neutralizing antibody titers were not observed in animals prior to vaccine administration or in saline-administered control animals (Table 2.6.2-4).

Table 2.6.2-4. Group Mean Titers of SARS-CoV-2 Neutralizing Antibodies

Interval/Occasion	Saline (0 µg RNA)	BNT162b2 (V9) (30 µg RNA)
Prior to Dosing Initiation	5.0	5.3
Just Prior to Mating	5.0	3886.4
Gestation Day 21 (Dams)	5.0	3445.5
Lactation Day 21	5.0	3620.4
Fetuses (Gestation Day 21)	5.0	640.0
Pups (Postnatal Day 21)	5.0	4561.4

2.6.2.8. Secondary Pharmacodynamics

No secondary pharmacodynamics studies were conducted with BNT162b2.

2.6.2.9. Safety Pharmacology

No safety pharmacology studies were conducted with BNT162b2 as they are not considered necessary according to the WHO guideline (WHO, 2005).

2.6.2.10. Pharmacodynamic Drug Interactions

Pharmacodynamic drug interaction studies with BNT162b2 have not been conducted.

2.6.2.11. Discussion and Conclusions

The BNT162b2 vaccine candidate encoding the full-length P2 S induces robust immune responses in mice, rats, and nonhuman primates. SARS-CoV-2 S is a primary target of neutralizing antibodies, and the modRNA that encodes the vaccine antigen induces a strong neutralizing antibody response, Th1-type $CD4^+$ T-cell response, and a $CD8^+$ IFNγ response. This diversity of elicited immune mechanisms could block virus infection as a first line of defense and clear virus-infected cells as a second line of defense.

A recombinant form of the P2 S antigen encoded by the vaccine and transiently expressed on the surface of mammalian cells was bound by a soluble ACE2 receptor and SARS-CoV-2 neutralizing monoclonal antibodies with high affinities. Analysis of the P2 S trimer structure by cryoelectron microscopy revealed high similarity to previously reported P2 S structures. The well-resolved trimeric prefusion structure and the high affinity binding to ACE2 and human neutralizing antibodies demonstrate that the recombinant full-length P2 S authentically presents the ACE2 binding site and other epitopes targeted by many SARS-CoV-2 neutralizing antibodies.

Nonclinical studies in mice and nonhuman primates showed that antigen-binding IgG and neutralizing antibody responses were detectable as early as 14 d post-immunization, with

substantial increases observed in nonhuman primates after the second dose. Similar results indicating immunogenicity were obtained in an accessory study to the GLP-compliant repeat-dose toxicology studies in rats (Study 38166 and Study 20GR142) and DART study (Study 20256434). In a SARS-CoV-2 rhesus challenge model, BNT162b2 provided partial protection from infection in the upper airway, and no viral RNA was detected in the lower airways, sampled serially by BAL starting 3 days after challenge. No evidence of disease enhancement was observed in BNT162b2-immunized and SARS-CoV-2 challenged macaques (VR-VTR-10671).

2.6.2.12. Immunogenicity and Efficacy Methods

2.6.2.12.1. SARS-CoV-2 S1 and RBD Direct ELISA

For preclinical studies in mice, antigen-based direct ELISAs measured S1-binding (S1 recombinant protein, Sino Biological) and RBD-binding (recombinant RBD, Sino Biological) IgG levels in serum samples. MaxiSorp plates (Thermo Fisher Scientific) were coated with recombinant protein (100 ng/100 µL) in sodium carbonate buffer, and bound IgG was detected using an HRP-conjugated secondary antibody and TMB substrate (Biotrend). Data collection was performed using a BioTek Epoch reader and Gen5 software version 3.0.9. For concentration analysis, the signal of the specific samples was correlated to a standard curve of an isotype control.

2.6.2.12.2. VSV/SARS-CoV-2 S Pseudovirus Neutralization Assay

For preclinical immunogenicity studies in rodents, a pseudotype neutralization assay (pVNT) was used as a surrogate of virus neutralization (which, for SARS-CoV-2, requires BSL3 containment). The pVNT is based on a recombinant replication-deficient vesicular stomatitis virus (VSV) vector that encodes GFP instead of VSV-G (VSVΔG-GFP). VSVΔG-GFP was pseudotyped with SARS-CoV-2 S protein according to published pseudotyping protocols (Berger & Zimmer 2011; Baum et al, 2020). Serial dilutions of mouse sera were incubated with the pseudotyped reporter virus for 10 minutes at room temperature before inoculating Vero-76 cell monolayers in 96 well plates. Virus was added at 300 IU per well and infected cell counts per well were detected 16-24 hours after inoculation with an IncuCyte Live Cell Analysis system (Sartorius) with IncuCyte 2019B Rev2 software. The 50% pseudovirus neutralization titer ($pVNT_{50}$) was reported as the reciprocal of the first serum dilution yielding a 50% reduction in GFP-positive infected cell number per well compared to the mean of the no serum pseudovirus positive control.

2.6.2.12.3. SARS-CoV-2 S1-Binding and RBD-Binding Kinetics using Surface Plasmon Resonance Spectroscopy

Binding kinetics of murine S1- and RBD-binding serum IgGs was determined using a Biacore T200 device (Cytiva). An anti-mouse-Fc antibody (Jackson ImmunoResearch) was covalently coupled to immobilization level of ~10,000 response units (RU) on the CM5 sensor chip matrix. Bulk mouse IgGs were captured from diluted serum and binding analyses to histidine-tagged S1 (S1-His) or histidine-tagged RBD (RBD-His) (Sino Biological) were performed using a multi-cycle kinetic method with concentrations ranging from 25 to 400 nM or 1.5625 to 50 nM, respectively. Binding kinetics were calculated using a global

kinetic fit to a 1:1 Langmuir model with Biacore T200 Evaluation Software Version 3.1 (Cytiva).

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

For nonhuman primate studies, 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.

2.6.2.12.5. SARS-CoV-2 Neutralization Assay

For nonhuman primate studies, the same authentic SARS-CoV-2 neutralization assay used for clinical testing was applied (VR-MQR-10214). 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 (Xie et al, 2020). This reporter virus generates similar plaque morphologies and indistinguishable growth curves from wild-type virus (Muruato et al, 2020). Viral master stocks used for the neutralization assay were grown in Vero E6 cells as previously described (Xie et al, 2020). Serial dilutions of heat inactivated sera were incubated with the reporter virus for 1 hour at 37 °C before inoculating Vero CCL81 cell monolayers in 96 well plates to allow accurate quantification of infected cells. Virus was added at 2 x 10^4 PFU per well to yield a target of 10-30% of infected cells in the monolayer. Total cell counts per well 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 was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.

2.6.2.12.6. ELISpot and Cytokine Profiling Immunoassays in Mice

Spleen single-cell suspensions were prepared in PBS by mashing tissue against the surface of a 70 µm cell strainer (BD Falcon). Erythrocytes were removed by hypotonic lysis. Popliteal, inguinal and iliac lymph nodes were pooled, cut into pieces, digested with collagenase D (1 mg/mL; Roche) and passed through cell strainers.

ELISpot assays were performed with mouse IFNγ ELISpot^{PLUS} kits according to the manufacturer's instructions (Mabtech). A total of 5 × 10^5 splenocytes was *ex vivo* restimulated with the full-length S peptide mix (0.1 µg/mL final concentration per peptide, JPT) or controls (gp70-AH1 [SPSYVYHQF] (Slansky et al, 2000), JPT). Streptavidin-ALP and BCIP/NBT-plus substrate were added, and spots counted using an ELISpot plate reader (ImmunoSpot® S6 Core Analyzer, CTL). Spot numbers were evaluated using

ImmunoCapture Image Aquision Software V7.0 and ImmunoSpot 7.0.17.0 Professional. For T-cell subtyping, $CD8^+$ T cells were isolated from splenocyte suspensions using MACS MicroBeads (CD8a [Ly-2], Miltenyi Biotec) according to the manufacturer's instructions. The flow-through served as a source of $CD4^+$ T cells. $CD8^+$ or $CD4^+$ T cells were subsequently restimulated with syngeneic bone marrow-derived dendritic cells loaded with full-length S peptide mix (0.1 µg/mL final concentration per peptide) or medium as control.

For cytokine profiling in mice by bead-based immunoassays, mouse splenocytes were re-stimulated for 48 h with full-length S peptide mix (0.1 µg/mL final concentration per peptide) or cell culture medium (no peptide) as control. Concentrations of IFNγ, IL-2, IL-4, IL-5 and (for splenocytes from BNT162b2-immunised mice) IL-13 in supernatants were determined using a bead-based, 11-plex TH1/TH2 mouse ProcartaPlex multiplex immunoassay (Thermo Fisher Scientific) according to the manufacturer's instructions. Fluorescence was measured with a Bioplex200 system (Bio-Rad) and analysed with ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific). Values below the lower limit of quantification (LLOQ) were set to zero.

2.6.2.12.7. ELISpot and Intracellular Cytokine Staining Assays in NHPs

Cryopreserved NHP 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.

For ELISpot assays, commercially available NHP IFNγ and IL-4 ELISpot assay kits were used (Mabtech, Sweden). Briefly, pre-coated PVDF 96-well microplates were washed 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. Cells were stimulated with a peptide pool spanning the entire S protein (15 mers, 11aa 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; media-DMSO, a CMV peptide pool (JPT, Germany) and PHA (Sigma, USA) were included as controls. Cells were removed, plates washed, and spots detected using a biotinylated detection antibody followed by a Streptavidin-HRP secondary antibody and AEC chromogenic substrate (BD, US) for 10 minutes for IFNγ and 30 minutes for IL-4 at room temperature until red spots were developed. Dried plates were scanned and counted using a CTL ImmunoSpot S6 Universal Analyzer (CTL, US). Reported results are background (media-DMSO) subtracted and normalized to spot forming cells (SFC)/10^6 PBMCs.

For intracellular cytokine staining (ICS) flow cytometry-based analysis, thawed PBMCs rested for 3 to 4 hours were stimulated in AIM-V medium in 96-well plates with the peptide pool spanning the entire S protein at 1 µg/mL; Staphylococcus enterotoxin B (SEB; 2 µg/mL) was used as a positive control; and 0.2 % DMSO was used as a negative control. An APC-conjugated CD107a monoclonal antibody, GolgiStop, and GolgiPlug were added to each well, and cells were incubated at 37 °C for 12 to 16 h. Cells were then stained with Viability Dye eFluor 780 and Fc block prior to surface staining with mAbs specific for CD4, and CD8. Following staining for surface markers, cells were fixed and permeabilized with BDCytoFix/CytoPerm solution, and intracellular staining performed with mAbs specific for the following proteins, diluted in permeabilization buffer: CD154, IFNγ, IL-2, IL-4, TNF-α, CD3. Cells were washed, resuspended in 2% fetal bovine serum (FBS)/ phosphate buffered

saline (PBS) buffer and acquired on a LSR Fortessa. Data were 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 (media-DMSO) subtracted.

2.6.2.12.8. Quantitative RT-PCR for Detection of SARS-CoV-2 Viral RNA

For quantification of SARS-CoV-2 virus in nonhuman primate challenge model swabs and bronchoalveolar lavage (BAL) specimens, the US Centers for Disease Control-developed 2019-nCoV_N1 assay, a sensitive reverse transcription-polymerase chain reaction (RT-PCR)-based assay that detects both viral genomic RNA and RNA transcripts, was used (Singh et al, 2020).

2.6.2.12.9. Lung Radiographs and Computed Tomography Scans

Lung radiographs (X-rays) and computed tomography (CT) scans were performed under anesthesia as previously described (Singh et al, 2020; Kaushal et al, 2015). 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 Days 3 and 6, for BNT162b2-immunized and control NHP 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”.

2.6.2.12.10. Macroscopic and Microscopic Pathology

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). 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 two pathologists, 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.

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2.6.3.1 Pharmacology: Overview

Test Article: BNT162b2

Type of Study	Test System	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics				
Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 S1 Protein in Human Sera	Direct Luminex immunoassay	NA	PWRD	VR-MQR-10211
Qualification of the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay	In vitro cell culture	NA	University of Texas Medical Branch (Galveston, TX)	VR-MQR-10214
BNT162b2 (V9) Immunogenicity and Evaluation of Protection against SARS-CoV-2 Challenge in Rhesus Macaques	Rhesus macaques	IM	PWRD New Iberia Research Center (New Iberia, LA) SNPRC (San Antonio, TX)	VR-VTR-10671
In Vitro Expression of BNT162b2 Drug Substance and Drug Product	In vitro cell culture	IM	BioNTech (Mainz, Germany)	R-20-0211
COVID-19: Immunogenicity Study Of The LNP-Formulated ModRNA Encoding The Viral S Protein-V9	BALB/c mice	IM	BioNTech (Mainz, Germany)	R-20-0085
Characterizing the Immunophenotype In Spleen And Lymph Node Of Mice Treated With SARS-CoV-2 Vaccine Candidates	BALB/c mice	IM	BioNTech (Mainz, Germany)	R-20-0112
Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen	In vitro cell culture	NA	PWRD	VR-VTR-10741
Secondary Pharmacodynamics				
Studies not conducted				
Safety Pharmacology				
Studies not conducted				
Pharmacodynamic Drug Interactions				
Studies not conducted				

COVID-19 – Coronavirus disease 2019; dLIA – Direct Luminex Assay; IgG – immunoglobulin G; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; NA – not applicable; PWRD – Pfizer Worldwide Research & Development; IM - intramuscular; SNPRC – Southwest National Primate Research Center.

MODULE 2.6.6 TOXICOLOGY WRITTEN SUMMARY

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

A:G	Albumin:globulin ratios
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BASO	Basophils
CBER	Center for Biologics Evaluation and Research
CoV	Coronavirus
COVID-19	Coronavirus Disease 2019
DART	Developmental and Reproductive Toxicology
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	Enzyme-linked immunosorbent assay
EOS	Eosinophils
F0	Parental generation administered vaccine
F1	First generation offspring of F0 generation
GD	Gestation day
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practice
HCT	Hematocrit
HGB	Hemoglobin
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
IM	Intramuscular(ly)
LNP	Lipid-nanoparticle
LUC	Large unstained cells
modRNA	Nucleoside-modified mRNA
MONO	Monocytes
mRNA	Messenger RNA
NEUT	Neutrophils
NHP	Nonhuman primate
OECD	Organisation for Economic Co-operation and Development
P2 S	Spike protein P2 mutant
PLT	Platelet
PND	Postnatal day
RBC	Red blood cells
RBD	Receptor binding domain
RETIC	Reticulocytes
RNA	Ribonucleic acid
S	SARS-CoV-2 spike glycoprotein
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2; coronavirus causing COVID-19

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

TBIL	Bilirubin, total
TNF	Tumor necrosis factor
V8	Variant 8; P2 S
V9	Variant 9; P2 S
WBC	White blood cells
WHO	World Health Organization

2.6.6. TOXICOLOGY WRITTEN SUMMARY

2.6.6.1. Brief Summary

Pfizer and BioNTech have developed a vaccine intended to prevent COVID-19 that is caused by SARS-CoV-2. The vaccine is based on RNA encoding the SARS-CoV-2 S glycoprotein antigen, which is formulated in LNP, and is referred to as BNT162b2 vaccine candidate (BioNTech code number BNT162, Pfizer code number PF-07302048).

The nonclinical toxicity assessment of the BNT162b2 vaccine candidate consists of 3 GLP-compliant studies in Wistar Han rats including 2 pivotal repeat-dose toxicity studies and a combined fertility and developmental study (Table 2.6.6-1 and Tabulated Summary 2.6.7.1). Multiple vaccine candidates were evaluated in the nonclinical safety studies; however, the focus will be on the results for BNT162b2 (V9), the vaccine advanced into the Phase 2/3 clinical trial and the subject of this application, and its variant BNT162b2 (V8), which was not administered clinically. BNT162b2 (V9) differs from BNT162b2 (V8) only in the optimized codons used to improve antigen expression, but the amino acid sequences of the encoded antigen are the same.

The design of the nonclinical repeat-dose toxicity studies was consistent with the WHO Guidelines on Nonclinical Evaluation of Vaccines, the EMA Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines, and Japan guidance on the nonclinical safety assessment of vaccines. In addition, the 2020 CBER guidance on "Development and Licensure of Vaccines to Prevent COVID-19" (US HHS, 2020) was considered when assembling the nonclinical safety licensure package as well as feedback from regulatory agencies. All GLP-compliant studies were conducted in accordance with Good Laboratory Practice for Nonclinical Laboratory Studies, Code of US Federal Regulations (21 CFR Part 58), in an OECD Mutual Acceptance of Data member state. All nonclinical studies described herein were conducted by or for Pfizer Inc or BioNTech RNA Pharmaceuticals GmbH. The location of records for inspection is included in each final study report.

Table 2.6.6-1. Overview of Toxicity Testing Program

Study[a]	Study (Sponsor) No.	Dose Group (µg RNA)	Total Volume (µL)[b]	No. of Animals/ Group	Tabulated Summary
Repeat-Dose Toxicity					
17-Day, 2 or 3 Dose (1 Dose/Week) IM Toxicity With a 3-Week Recovery Phase in Rats	38166	Control[c] (0)	200[e]	15/sex	2.6.7.7A
		BNT162a1 (30)	60	15/sex	
		BNT162a1 (30)	20	15/sex	
		BNT162b1 (30)	60	15/sex	
		BNT162b1 (100)	200[e]	15/sex	
		BNT162c1 (30)	70	15/sex	
		BNT162b2 (V8)[d] (100)	200[e]	15/sex	
17-Day, 3 Dose (1 Dose/Week) IM Toxicity With a 3 Week Recovery Phase in Rats	20GR142	Saline[f] (0)	60	15/sex	2.6.7.7B
		BNT162b2 (V9)[d] (30)	60	15/sex	
		BNT162b3[g] (30)	60	15/sex	
Reproductive & Developmental Toxicity					
IM Combined Fertility and Developmental (Including Teratogenicity and Postnatal Investigations) Toxicity in Rats	20256434 (RN9391 R58)	Saline[f] (0)	60	44 F	2.6.7.12
		BNT162b1 (30)	60	44 F	
		BNT162b2 (V9)[d] (30)	60	44 F	
		BNT162b3 (30)	60	44 F	

a. All studies are GLP-compliant and were conducted in an OECD mutual acceptance of data-compliant member state.
b. Doses were administered as 1 application at 1 site unless otherwise indicated.
c. Phosphate buffered saline, 300 mM sucrose.
d. Bold text highlighting the BNT162b2 vaccine candidates.
e. One application (100 µL) at 2 sites for a total dose volume of 200 µL.
f. Sterile saline (0.9% NaCl).
g. BNT162b3 is also referred to as BNT162b3c in study reports.

In the repeat-dose toxicity studies, 30 or 100 µg BNT162b2 was tolerated when administered once weekly for a total of 3 IM doses. There were no vaccine-related clinical signs or mortalities observed. The vaccine induced an inflammatory response which manifested as increases in typical inflammatory blood parameters such as fibrinogen, acute phase proteins, white blood cells (including NEUT, EOS, BASO, MONO, and/or LUC), local injection site reactions, transient increases in body temperature compared with controls, and microscopic inflammation at the injection site, which sometimes extended into the surrounding tissues.

Effects considered secondary to immune activation and the inflammatory response included a reversible reduction in body weight post immunization without affecting body weight gain between immunizations, transient decreases in RETIC, minimal decrease in RBC mass parameters, and slight decreases in PLT. Evidence of an immune response was observed not only in antigen-binding IgG and serum neutralizing response, but also as enlargement and increased cellularity of germinal centers in the draining (iliac) lymph node. Responses to inflammation were manifested as increased cellularity in the bone marrow and increased extramedullary hematopoiesis in the spleen, which were associated with macroscopic increased spleen size and increased absolute and relative spleen weight.

There were two vaccine-related nonadverse observations relevant to the liver. First, plasma activity of GGT was elevated in comparison to the control group. There was no elevation in ALP or TBIL and no macroscopic or microscopic findings consistent with cholestasis or hepatobiliary injury. Of note, elevation of GGT was not replicated with BNT162b2 (V9) in the second repeat-dose toxicity study (Study 20GR142). Second, a nonadverse, reversible vacuolation of portal hepatocytes was present in animals administered BNT162b2, which was not associated with alterations in hepatic function (eg, no elevations in ALT or AST). This change may be related to hepatic distribution of the lipids in the LNP (Sedic et al, 2018).

No new findings were observed during the recovery phase. At the end of the recovery, all vaccine induced effects on local tolerance and body weight were fully reversed and most clinical pathology parameter changes had resolved. Macroscopic and microscopic findings had partial or complete recovery, although some animals treated with BNT162b2 still had enlarged iliac lymph nodes and minimal to mild inflammation observed microscopically at the injection site at the end of the recovery phase.

In the combined fertility and developmental study, administration of 4 IM doses (twice before mating and twice during gestation) of BNT162b2 at 30 µg RNA/dosing day was associated with nonadverse effects (body weight, food consumption and effects localized to the injection site) after each dose administration in F0 female rats. There were no BNT162b2-related effects on mating performance or fertility in F0 female rats or on embryo-fetal or postnatal survival, growth, or development of the F1 offspring.

2.6.6.1.1. Test Article

The BNT162b2 vaccine is a preservative-free, sterile dispersion of RNA formulated in LNP in aqueous cryoprotectant buffer for IM administration.

BioNTech has developed an RNA vaccine platform which utilizes nucleoside-modified mRNA (modRNA) with blunted innate immune activating capacity and augmented antigen expression. These modRNA-based vaccines are formulated in LNPs and encode the SARS-CoV-2 P2 mutant S glycoprotein (P2 S). Each candidate is also given a V number that indicates the specific version of the optimized insert genomic sequence but still coding for the same antigen. The 2 related variants of BNT162b2 evaluated in the repeat-dose toxicity studies are described below:

- BNT162b2 (V9) (RBP020.2): modRNA encoding the SARS-CoV-2 full-length, P2 mutant, prefusion-stabilized spike glycoprotein (P2 S) (V9) - final candidate

- BNT162b2 (V8) (RBP020.1): modRNA encoding the SARS-CoV-2 full-length, P2 mutant, prefusion-stabilized spike glycoprotein (P2 S) (V8) – related variant

Doses up to 100 µg RNA/dose of the BNT162b2 vaccine candidate have been evaluated in the clinic. The dose of BNT162b2 (V9) selected for licensure is 30 µg RNA/dose.

Each vaccine is formulated in an LNP containing 4 lipids: ALC-0315, ALC-0159, DSPC, and cholesterol. Other excipients in the formulation include sucrose, NaCl, KCl, Na_2HPO_4, and KH_2PO_4.

Either saline or a solution of phosphate-buffered saline with 300 mM sucrose was used to dose animals that received buffer control.

IM administration was chosen as this is the clinical route of administration. Doses of BNT162b2 (V8 or V9) were administered in the nonclinical safety studies as one 60 or two 100 µL injections/dosing day (at 30 or 100 µg RNA, respectively) into the left and/or right quadriceps muscles. RNA concentrations for the BNT162b2 (V8 and V9) batches used in the repeat-dose toxicity and DART studies were approximately 0.5 mg/mL.

2.6.6.1.2. Animals

Rats were selected as the species for assessing the toxicity of the BNT162b2 vaccine as they demonstrated an immune response to the BNT162b2 vaccine antigen (Section 2.6.2.7) and are a commonly used species in toxicity studies with a large historical database.

Wistar Han rats supplied by Charles River Laboratories (Germany) GmbH were used in the repeat-dose toxicity study (Study 38166) with BNT162b2 (V8). Wistar Han rats supplied by Charles River Laboratories (USA) were used in the repeat-dose toxicity study (Study 20GR142) with BNT162 (V9). Wistar Han rats supplied by Charles River Laboratories (France) were used in the combined fertility and developmental study (Study 20256434).

2.6.6.2. Single-Dose Toxicity

A separate single-dose toxicity study with the BNT162b2 vaccine candidate has not been conducted.

2.6.6.3. Repeat-Dose Toxicity

2.6.6.3.1. Repeat-Dose Toxicity Study of Three LNP-Formulated RNA Platforms Encoding for Viral Proteins by Repeated Intramuscular Administration to Wistar Han Rats

The objective of this pivotal repeat-dose toxicity study was to determine the potential toxicity of three LNP--formulated RNA vaccine platforms, encoding SARS-CoV-2 P2 S or RBD, administered once weekly by IM administration to rats and to assess the reversibility of any effects after a 3-week recovery phase (Study 38166; Tabulated Summary 2.6.7.7A). The LNP

formulation was the same for the three RNA platforms administered in this study. As the vaccine candidate selected for licensure is BNT162b2 (V9), the summary of the results described below will focus only on the closely related variant, BNT162b2 (V8), which was evaluated in this study. However, overall findings were similar among the vaccine candidates evaluated with the 3 RNA platforms. Details on the findings with the other vaccine candidates evaluated can be found in the study report.

Wistar Han rats (15/sex/group) were administered doses of 0 (buffer) or 100 µg RNA/dose/animal BNT162b2 (V8) via IM injection. Doses were administered once a week for 3 weeks (Days 1, 8, 15). The dose volume was 200 µL/dosing day (100 µL injected into each hindlimb). Following the dosing phase, 10 animals/sex from each group were euthanized 2 days post the last immunization for post-mortem assessments. The remaining 5 animals/sex/group were euthanized following a 3-week recovery phase. Additional satellite animals (3/sex/group) were used for blood sampling for cytokine analysis.

Clinical signs of toxicity were assessed twice daily throughout the study. Body weights were recorded twice weekly during the dosing and the recovery phase. Food consumption was evaluated once weekly. Local tolerance (injection site dermal assessment) was evaluated after each administration, and body temperatures were evaluated at 4 and 24 hours after each administration. Serum cytokines (IFN-γ, TNF-α, IL-1β, IL-6, IL-10) were evaluated prior to and 6 hours post each dose and at the end of the dosing phase. Clinical pathology (hematology and clinical chemistry parameters as well as acute phase proteins) was evaluated 3 days after the first administration and at the end of the dosing and recovery phases. Urinalysis, coagulation parameters, auditory and ophthalmological parameters, serology, organ weights, macroscopic and microscopic pathology were evaluated at the end of dosing and recovery phases.

IM administration of BNT162b2 (V8) once weekly for 3 administrations to male and female Wistar Han rats was tolerated without evidence of systemic toxicity and produced the expected local inflammatory reaction.

No test article-related unscheduled euthanasias or deaths occurred during the study. There were no test-item related ophthalmologic or auditory alterations exhibited. There were also no test article-related systemic changes in behavior, external appearance, or consistency of feces.

Clinical findings included transient decreases in mean body weight and transient increases in mean body temperature. The mean body weight of the BTN162b2 (V8) group was transiently decreased after each administration compared with predose values (down to 0.92x) but were close to comparable to controls by the end of recovery. The mean body temperature of the BTN162b2 (V8) group was transiently higher at 4 and/or 24 hours after each administration compared with the control group. There were no test item effects on body weight or body temperature during the recovery phase.

Test article-related injection site observations included edema and erythema; with edema being the most common finding. After the first administration, most animals (23 of 30) administered BNT162b2 (V8) developed very slight edema or rarely, slight erythema. The

incidence of injection site observations was higher and the observations were more severe (up to moderate edema or more rarely severe edema or erythema) after the second and third dose administration compared with the first administration. However, all observations resolved prior to the subsequent dosing and were fully recovered at the end of the 3-week recovery phase. The occurrence of higher severity local reactions after boost immunizations was attributed to the short immunization interval and to the high vaccine dose, in relation to the bodyweight of the rat (approximately up to 0.5 mg/kg). Macroscopic findings at the injection sites included induration or thickening, which was noted for 16 of 20 BNT162b2 (V8)-treated animals at the end of the dosing phase. This correlated microscopically with mild to marked inflammation in all BNT162b2 (V8)-administered animals at the end of the dosing phase. Inflammation was mixed to mononuclear (characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis) with fibrosis, minimal to marked edema, and minimal to mild myofiber degeneration (very rarely, minimal necrosis). Inflammation was occasionally evident extending into tissues adjacent to the injection site (including perineural tissue of sciatic nerve, tissue around the femur/knee and to the draining [iliac] lymph node) and was accompanied by elevations in circulating WBC (up to 2.2x controls), NEUT (up to 7.8x controls), EOS (up to 6.1x controls), BASO (up to 2.5x controls), and LUC (up to 7.7x controls) and acute phase proteins (fibrinogen [up to 3.1x controls], alpha-2-macroglobulin [up to 217x of controls], and alpha-1-acid glycoprotein [up to 21x of controls]). Consistent with an acute phase response (Sellers et al, 2020), lower plasma albumin (down to 0.87x controls) and higher plasma globulin (up to 1.2x controls), resulting in an altered A:G ratio, were observed in BNT162b2 (V8)-dosed animals. The findings were typical of an inflammatory response to LNP-encapsulated mRNA vaccines. The injection site findings were not interpreted as adverse because of lack of systemic toxicity and absence of clinical signs of lameness.

Effects considered secondary to immune activation/acute phase responses and inflammation at the injection site included transient lower RETIC (down to 0.28x controls; Day 4 only), minimal lower red cell mass parameters (RBC, HGB, and HCT; down to 0.87x controls) on Day 17 only, and sporadic lower PLT (down to 0.66x controls), which were small in magnitude. PLT reductions were likely due to inflammation-related PLT activation and consumption and were unassociated with alterations in hemostasis.

At the end of the 3-week recovery phase, all clinical injection site findings, clinical pathology findings, and macroscopic observations described above had resolved and there was evidence of recovery of the injection site inflammation microscopically.

Test article-related macroscopic enlargement of the draining (iliac) lymph nodes was evident at the end of dosing. Microscopically, this finding correlated with mild to moderate increased cellularity of germinal centers and mild to moderate increased plasma cells in the draining (iliac) lymph node and is an anticipated immune response to the administered vaccine and LNP. At the end of the 3-week recovery phase, a few animals administered BNT162b2 (V8) still had slightly enlarged iliac lymph nodes. All other BNT162b2 (V8)-related changes in the draining lymph node had resolved.

Test article-related macroscopic enlargement of spleen and associated absolute and relative (to body weight) spleen weights (up to 1.7x controls) correlated microscopically to minimal to mild increased hematopoiesis. Minimal increased hematopoiesis was also evident in the bone marrow. Both findings were fully resolved at the end of the 3-week recovery phase.

Test article-related microscopic vacuolation of portal hepatocytes (minimal to mild) was present in most animals (19 of 20) administered BNT162b2 (V8) at the end of the dosing phase. This finding was not adverse because it was unassociated with alterations in hepatic function (eg, no elevations in ALT or AST) and was fully reversed at the end of the 3-week recovery phase. This change may be related to hepatic distribution of the lipids from the LNP (Sedic et al, 2018).

Higher GGT (up to 4.6x controls), which is a biomarker of biliary, not hepatocellular injury (Boone et al, 2005), was evident in all BNT162b2 (V8)-administered animals on Days 4 and/or 17. There were no other hepatobiliary biomarker alterations or macroscopic or microscopic findings consistent with cholestasis or hepatobiliary injury to explain the higher level of GGT, which was completely resolved at the end of the 3-week recovery phase.

No BNT162b2 (V8)-related changes were observed for cytokine serum concentrations or in urinalysis parameters.

Immunogenicity assessment demonstrated that BNT162b2 (V8) elicited a SARS-CoV-2 S -binding IgG response directed against the S1 fragment and the RBD. Antibody responses detected via ELISA correlated with neutralizing activity as seen in the pseudovirus neutralization test with BNT162b2 (V8) eliciting higher antigen-binding IgG levels and also higher pseudovirus neutralization titers. Further details can be found in Section 2.6.2.7.

In conclusion, administration of BNT162b2 (V8) via IM injections once weekly for 3 administrations to male and female Wistar Han rats was tolerated without evidence of systemic toxicity, elicited a robust antigen-specific immune response, and produced nonadverse inflammatory changes at the injection sites and the draining lymph nodes, increased hematopoiesis in the bone marrow and spleen, and clinical pathology changes consistent with an immune response or inflammation at the injection sites. There was nonadverse minimal hepatocellular vacuolation in periportal regions of the liver that may be related to hepatic distribution of the lipid in the LNP. The findings in this study were nonadverse, reversible, and consistent with those typically associated with the IM administration of LNP-encapsulated mRNA vaccines (Hassett et al, 2019).

2.6.6.3.2. 17-Day Intramuscular Toxicity Study of BNT162b2 (V9) in Wistar Han Rats With a 3-Week Recovery

The objectives of this pivotal repeat-dose toxicity study were to determine the potential toxicity and development of a specific immune response to the antigens in each of the vaccine candidates, BNT162b2 (V9) and BNT162b3c, administered once weekly by IM injection for a total of 3 doses to Wistar Han rats (Study 20GR142; Tabulated Summary 2.6.7.7B). The reversibility of potential effects were evaluated following a 3-week recovery phase. As the vaccine candidate selected for licensure was BNT162b2 (V9), the summary of the results described below will focus on only that candidate. However, overall

findings were similar between the two candidates. Details on the findings with the other vaccine candidate evaluated in this study, BNT162b3c, can be found in the study report.

Wistar Han rats (15/sex/group) were administered IM doses of 0 (saline) or 30 µg RNA/dose/animal BNT162b2 (V9). Doses were administered once a week for 3 weeks (Days 1, 8, 15) at a dose volume of 60 µL/dose. Following the dosing phase, 10 animals/sex from each group were euthanized 2 days post last immunization for post-mortem assessments. The remaining 5 animals/sex/group were euthanized following a 3-week recovery phase.

Clinical signs were assessed twice daily throughout the study. Body weights were recorded twice prior to the initiation of dosing, predose on Days 1, 8, and 15, and on Days 4 and 11, twice weekly during the recovery phase and just prior to scheduled necropsy. Food consumption was evaluated on Days 4, 8, 11, and 15 and twice weekly during the recovery phase. Local tolerance (injection site dermal assessment) was evaluated 4 and 24 hours after each administration and at 72 hours post-last dose for recovery animals. Additional injection site assessments 48 and 72 hours post injection were collected for animals that had a score of 2 or greater at 24 hours. Body temperature measurements were taken predose on Days 1, 8, and 15 and again at 4 and 24 hours postdose. Clinical pathology (hematology, clinical chemistry parameters, as well as acute phase proteins) was evaluated on Days 4 and 17 and at the end of the recovery phase. Urinalysis, coagulation parameters, and ophthalmological parameters, serology, organ weights, macroscopic and microscopic pathology were evaluated at the end of dosing and recovery phases.

There was no unscheduled euthanasia. All animals administered BNT162b2 (V9) survived to scheduled necropsy at the end of the dosing or recovery phase of the study. There were no vaccine-related clinical signs observed, or changes to urinalysis or ophthalmoscopic parameters during the dosing phase of the study.

Test article-related lower mean food consumption (down to 0.83x controls) was noted on Days 4 and 11 for animals receiving BNT162b2 (V9). Test article-related higher mean food consumption (1.08x-1.35x control) was noted throughout the recovery phase for male animals administered BNT162b2 (V9). No test article-related mean body weight changes were noted for animals administered BNT162b2 (V9) during the dosing phase. Test article-related higher mean body weight (1.05x-1.06x control) was noted in males only on Recovery Days 11, 15, 18, and 21 for animals administered BNT162b2 (V9).

Test article related higher mean body temperature (maximum increase post each dose) compared with concurrent control was noted on Days 1 (up to 0.54°C), 8 (up to 0.98°C) and 15 (up to 1.03°C) post dose administration of BNT162b2 (V9). No animal had a body temperature above 40°C through the dosing phase of the study.

BNT162b2 (V9)-related injection site edema Grade 2 (slight, edges of area well defined by definite raising) or Grade 3 (moderate, raised approximately 1 mm) were noted in most animals and occurred following dosing on Days 1, 8 and/or 15. The edema was generally observed up to 72 hours postdose and fully resolved. Erythema was also observed at the injection site in most animals following each dose administration; however, it was only a

Grade 1 (very slight, barely perceptible) and fully resolved prior to the next dose administration and after the last administration.

BNT162b2 (V9)-related changes in clinical pathology parameters included higher WBC and fibrinogen and lower A:G ratios, RETIC, RBC mass parameters. Higher WBC (up to 2.64x controls), primarily involving NEUT (up to 6.60x controls), MONO (up to 3.30x controls), and LUC (up to 13.2x controls) but also affecting EOS (up to 3.17x controls) and BASO (up to 8.00x controls) were present on Days 4 and 17, with higher values on Day 17. Lower A:G ratios (down to 0.82x controls; with associated but more variable lower total proteins and albumin [down to 0.92x and 0.85x controls, respectively] and/or higher globulin [up to 1.10x controls]) were observed on Days 4 and 17. Higher fibrinogen occurred on Day 17 (up to 2.49x controls), consistent with an acute phase response. The acute phase proteins alpha-1-acid glycoprotein (up to 39x controls on Day 17) and alpha-2 macroglobulin (up to 71x controls on Day 17) were elevated in both males and females in the BNT162b2 (V9)-administered group on Days 4 and 17 with higher concentrations generally observed in males. Transiently lower RETIC were present on Day 4 (down to 0.27x controls) and higher RETIC were present on Day 17 (1.31x controls; females only). Lower RBC mass parameters (RBC, HGB, HCT; up to 0.90x controls) were present on Days 4 and 17. All test article-related clinical pathology changes noted in the dosing phase were fully reversed after a 3-week recovery phase, with the exception of higher red cell distribution width, higher globulins, and lower A:G ratio (females) administered BNT162b2(V9).

There were test article-related higher spleen weights, macroscopic observations of enlarged draining (iliac) lymph nodes, and discolored or firm injection sites. Test article-related higher group mean absolute and relative (to body and brain weight) spleen weights were present in males (up to 1.42x controls) and females (up to 1.62x controls) administered BNT162b2 (V9). Test article-related macroscopic findings included the observation of large draining lymph nodes (abnormal size, enlarged; 1 of 10 males and 1 of 10 females) and pale/dark or firm injection sites (abnormal color, dark/pale and abnormal consistency, 2 of 10 males and 3 of 10 females; firm, 2 of 10 males and 4 of 10 females) in animals administered BNT162b2 (V9). At the end of recovery, no test article-related organ weight changes were noted and macroscopic findings were limited to large draining lymph nodes (abnormal size, enlarged) indicating a partial recovery of these findings. Pale/dark and/or firm injection sites and enlarged spleen were not observed at the end of recovery phase indicating a complete recovery of these findings.

At the conclusion of the dosing phase, nonadverse test article-related microscopic findings consistent with immune activation and an inflammatory response included mixed cell inflammation and edema of the injection sites (which correlated with macroscopic observations of abnormal color, dark/pale and abnormal consistency, firm), increased cellularity of plasma cells and germinal centers of the draining (iliac) and inguinal lymph nodes (which correlated with macroscopic observation of abnormal size of iliac, enlarged), increased cellularity of hematopoietic cells and germinal centers of the spleen (which correlated with macroscopic observation of abnormal size, enlarged and increased spleen weights), and increased cellularity of hematopoietic cells in the bone marrow were noted. These test article-related changes fully recovered, except for partial recovery of enlarged draining (iliac) lymph nodes and microscopic findings of inflammation at the injection sites,

increased cellularity of plasma cells and germinal centers in the draining and inguinal lymph nodes and increased cellularity of the germinal centers in the spleen.

In addition, test article-related vacuolation of the periportal hepatocytes in the liver was observed, in the absence of biochemical evidence of liver injury, and may be related to hepatic distribution of LNP lipids (Sedic et al, 2018). At the end of the 3-week recovery phase, this finding was completely recovered.

Administration of 3 once weekly doses of BNT162b2 (V9) elicited SARS-CoV-2 neutralizing antibody responses in both males and females at the end of the dosing and recovery phases of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals.

In conclusion, BNT162b2 (V9) administered via IM injection once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats was tolerated without evidence of systemic toxicity, generated a SARS-CoV-2 neutralizing antibody response, and produced nonadverse changes consistent with an immune or inflammatory response at the conclusion of the dosing phase. At the end of the 3-week recovery phase, full or partial recovery of all findings was observed. Other nonadverse findings included vacuolation in the liver which may be related to hepatic distribution of LNP lipids and was noted at the conclusion of the dosing phase and completely recovered. The findings in this study are consistent with those typically associated with the IM administration of LNP-encapsulated mRNA vaccines. Animals administered BNT162b2 (V9) elicited SARS-CoV-2 neutralizing antibody responses at the end of the dosing and recovery phases of the study.

2.6.6.4. Genotoxicity

No genotoxicity studies are planned for BNT162b2, as the components of all vaccine constructs are lipids and RNA that are not expected to have genotoxic potential (WHO, 2005).

2.6.6.5. Carcinogenicity

Carcinogenicity studies with BNT162b2 have not been conducted as the components of all vaccine constructs are lipids and RNA that are not expected to have carcinogenic or tumorigenic potential. Carcinogenicity testing is generally not considered necessary to support the development and licensure of vaccine products for infectious diseases (WHO, 2005).

2.6.6.6. Reproductive and Developmental Toxicity

Overall, there were no effects of BNT162b2 administration on female fertility, pregnancy, or embryo-fetal or offspring development. In addition, macroscopic and microscopic evaluation of male and female reproductive tissues from the repeat-dose toxicity studies with BNT162b2 showed no evidence of toxicity.

2.6.6.6.1. A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by Intramuscular Administration in the Wistar Han Rat

BNT162b2 (V9) was administered by IM injection at the human clinical dose (30 µg RNA/dosing day) to 44 female Wistar Han rats (F0) 21 and 14 days prior to mating with untreated males and on GDs 9 and 20, for a total of 4 dosing days (Study 20256434). A separate control group of 44 F0 females received saline by the same route and regimen. This study also included assessment of two other LNP-formulated RNA vaccine candidates (BNT162b1 and BNT162b3) that did not proceed into Phase 2/3 clinical trials. Here, the study findings from BNT162b2 are summarized; findings from the BNT162b1 and BNT162b3 vaccine candidates also tested in this study were generally similar and can be found in the study report.

Following completion of a mating phase with untreated males, 22 rats/group underwent caesarean-section on GD 21 and were submitted to routine embryo-fetal development evaluations. The remaining 22 rats/group were allowed to litter and behavior of the mothers and development of the offspring was observed until PND 21.

There were no BNT162b2-related deaths during the study. IM administration of BNT162b2 before and during gestation to female Wistar rats resulted in non-adverse 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. These maternal findings are all consistent with administration of a vaccine and an inflammatory/immune response and with those observed in the repeat-dose toxicity studies with BNT162b2.

There were no BNT162b2-related effects on any mating or fertility parameters. There were no BNT162b2-related effects on any ovarian, uterine, or litter parameters, including embryo-fetal survival, growth, or external, visceral, or skeletal malformations, anomalies, or variations. There were no effects of BNT162b2 administration on postnatal offspring (F1) development, including postnatal growth, physical development (pinna unfolding and eye opening), neurodevelopment (pre-weaning auditory and visual function tests), macroscopic observations, and survival.

All of F0 females administered BNT162b2 developed a SARS-CoV-2 neutralizing antibody response and these responses were detectable in all fetuses and pups from the caesarean and littering groups, respectively. The animals in the saline control group did not exhibit an immune response to BNT162b2.

In conclusion, administration of BNT162b2 (V9) to female rats twice before the start of mating and twice during gestation at the human clinical dose was associated with non-adverse effects (body weight, food consumption and effects localized to the injection site) after each dose administration. However, there were no effects of BNT162b2 administration on mating performance, fertility, or any ovarian or uterine parameters in the F0 female rats nor on embryo-fetal or postnatal survival, growth, or development in the F1 offspring. An immune response was confirmed in F0 female rats following administration of BNT162b2 and these responses were also detectable in the F1 offspring (fetuses and pups).

2.6.6.7. Local Tolerance

Local tolerance of IM administration of BNT162b2 was evaluated by injection site observations and macroscopic and microscopic examination of injection sites in the pivotal repeat-dose toxicity studies and are described above (Section 2.6.6.3).

2.6.6.8. Other Toxicity Studies (if available)

2.6.6.8.1. Antigenicity

Immunogenicity was evaluated as part of the primary pharmacology studies (Sections 2.6.2.5 and 2.6.2.6). In general, administration of BNT162b2 variants (V8 and V9) generated a robust immune response in non-GLP mouse and NHP immunogenicity studies. Serology data from the repeat-dose toxicity studies and the DART study showed a robust antigen-specific immune response to BNT162b2 (2.6.2.7).

2.6.6.8.2. Immunotoxicity

Stand-alone immunotoxicity studies with BNT162b2 have not been conducted. However, immunotoxicological endpoints have been collected as part of the pivotal repeat-dose toxicity studies. There were no adverse effects observed and no significant effects on measured cytokines.

2.6.6.9. Discussion and Conclusions

Administration of BNT162b2 by IM injection to male and female Wistar Han rats once every week for a total of 3 weekly cycles of dosing was tolerated without evidence of systemic toxicity in GLP-compliant repeat-dose toxicity studies. Expected inflammatory responses to the vaccine were evident such as edema and erythema at the injection sites, transient elevation in body temperature, elevations in WBCs and acute phase reactants, and lower A:G ratios. A transient elevation in GGT was noted in animals administered BNT162b2 (V8) in Study 38166 without evidence of microscopic changes in the biliary system or other hepatobiliary biomarkers but was not recapitulated in Study 20GR142. Injection site reactions were common in all vaccine-administered animals and were greater after boost immunizations. Changes secondary to inflammation included slight and transient reduction in body weights and transient reduction in RETIC, PLT, and RBC mass parameters. All changes in clinical pathology parameters and acute phase proteins were reversed at the end of the recovery phase for BNT162b2 with the exception of higher red cell distribution width, higher globulins, and lower A:G ratios in animals administered BNT162b2 (V9). Macroscopic pathology and organ weight changes were also consistent with immune activation and inflammatory response and included increased size of draining iliac lymph nodes and increased size and weight of spleen. Vaccine-related microscopic findings at the end of the dosing phase consisted of edema and inflammation in injection sites and surrounding tissue; increased cellularity in the draining iliac and inguinal lymph nodes, bone marrow, and spleen; and hepatocyte vacuolation in the liver. Periportal vacuolation of hepatocytes was not associated with any microscopic evidence of hepatic injury or alterations in liver function tests and is interpreted to reflect hepatocyte uptake of the LNP lipids (Sedic et al, 2018). Microscopic findings at the end of the dosing phase were partially or completely recovered in all animals at the end of the recovery phase for BNT162b2. A robust immune response was elicited to the BNT162b2 antigen.

Administration of BNT162b2 (V9) to female rats twice prior to mating and twice during gestation resulted in maternal observations (local reactions, transient decreases in body weight and food consumption) similar to those seen in the repeat-dose toxicity studies. However, there were no BNT162b2-related effects on female fertility, pregnancy, or embryo-fetal or offspring development in the presence of SARS-CoV-2 neutralizing antibodies in the maternal animals, fetuses, and pups. This is consistent with the observation of no macroscopic or microscopic findings in reproductive organs in the repeat-dose toxicity studies.

The results of the rat repeat-dose toxicity studies with the BNT162b2 variants (V8 and V9) and DART study with BNT162b2 (V9) demonstrate tolerability of the COVID-19 vaccine. Given the lack of adverse findings in the rats related to COVID-19 vaccine administration, the nonclinical toxicity program supports the clinical administration of BNT162b2 twice by IM injection at a dose of 30 µg RNA.

2.6.6.10. References

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2.6.7.1 Toxicology: Overview

Test Article: BNT162b2

Type of Study	Species/ Strain	Method of Administration	Duration of Dosing	Dose (µg RNA/animal)	Total Volume (µL)[a]	GLP Compliance	Testing Facility	Study Number (Sponsor Reference Number)
Single-Dose Toxicity								
Not conducted								
Repeat-Dose Toxicity								
17-Day, 2- or 3-Dose (1 Dose/Week) Toxicity With a 3-Week Recovery Phase	Rat/ Wistar Han	IM Injection	17 Days (Dose days 1, 8, 15) or 10 Days (Dose days 1, 8)[b]	0 (Control Buffer[c])	200[d]	Yes	(b) (4)	38166 (NA)
				30 (BNT162a1)	60			
				10 (BNT162a1)	20			
				30 (BNT162b1)	60			
				100 (BNT162b1)	200[d]			
				30 (BNT162c1)	70			
				100 (BNT162b2 [V8])	200[d]			
17-Day, 3-Dose (1 Dose/Week) Toxicity With a 3-Week Recovery Phase	Rat/ Wistar Han	IM Injection	17 Days (Dose days 1, 8, 15)	0 (Saline)[f]	60	Yes	PWRD[g]	20GR142 (NA)
				30 (BNT162b2 [V9])	60			
				30 (BNT162b3)	60			
Genotoxicity								
Not conducted								
Carcinogenicity								
Not conducted								

2.6.7.1 Toxicology: Overview

Test Article: BNT162b2 (continued)

Type of Study	Species/ Strain	Method of Administration	Duration of Dosing	Dose (µg RNA/animal)	Total Volume (µL)[a]	GLP Compliance	Testing Facility	Study Number (Sponsor Reference Number)
Reproductive and Developmental Toxicity								
Combined Fertility and Developmental (Including Teratogenicity and Postnatal Investigations)	Rat/ Wistar Han	IM Injection	21 and 14 days prior to mating, GD 9, GD 20	0 (Saline)[f] 30 (BNT162b1) 30 (BNT162b2 [V9]), 30 (BNT162b3)	60 60 60 60	Yes	Charles River Laboratories[h]	20256434 (RN9391 R58)
Local Tolerance								
Not conducted								
Other Toxicity Studies								
Not conducted								

GD = Gestation day; GLP = Good Laboratory Practice; IM = Intramuscular; (b) (4); NA = Not applicable; PWRD = Pfizer Worldwide Research & Development; QW = Once weekly.

a. Doses were administered as 1 application at 1 site unless otherwise indicated.

b. QWx3 (Days 1, 8, 15) for BNT162a1, BNT162b1, and BNT162b2 (V8); QWx2 (Days 1, 8) for BNT162c1.

c. Phosphate buffered saline, 300 mM sucrose.

d. One application (100 µL) at 2 sites for a total dose volume of 200 µL.

(b) (4)

f. Sterile saline (0.9% NaCl).

g. Groton, CT, US.

h. Saint-Germain-Nuelles, France.

2.6.7.7A Repeat-Dose Toxicity

Report Title: Repeat-Dose Toxicity Study of Three LNP-Formulated RNA Platforms Encoding for Viral Proteins by Repeated Intramuscular Administration to Wistar Han Rats

Test Article: BNT162b2

Species/Strain: Rat/Wistar Han

Age at First Dose: ~8-9 Weeks
Date of First Dose: 17 March 2020

Duration of Dosing: QWx3 (Days 1, 8, 15) for BNT162a1 (uRNA-LNP RBD), BNT162b1 (modRNA-LNP RBD), or BNT162b2 [V8] (modRNA-LNP SP2); QWx2 (Days 1, 8) for BNT162c1 (saRNA-LNP RBD)
Duration of Postdose: 3 Weeks
Method of Administration: Intramuscular injection; 20 to 100 µL/administration site/dose[b]

Study Number: 38166[a]
Lot Numbers: CoVVAC/090320 (BNT162a1), CoVVAC/100320 (BNT162b1), CoVVAC/130320 (BNT162c1), CoVVAC/160320 (BNT162b2 [V8])
GLP Compliance: Yes

Vehicle/Formulation: Phosphate buffered saline, 300 mM sucrose/Solution
Special Features: Cytokine analysis (IFN-γ, TNF-α, IL-1β, IL-6, IL-10)
No Observed Adverse Effect Level: 30 µg (BNT162a1, BNT162c1), 100 µg (BNT162b1, BNT162b2)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of Animals[c]	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Noteworthy Findings Died or Euthanized Moribund	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Body Weight (g)[d]														
Prior to Initiation of Dosing	257.6	213.8	1.0	1.0	1.0	0.89	1.0	1.0	1.0	0.90	1.0	0.91	1.0	0.90
Day 1	263.5	212.3	0.99	0.99	1.2†	1.0	1.0	1.0	1.2†	1.0	1.2†	1.0	1.2†	1.0
Day 2	268.9	215.1	0.93†	0.95	1.1†	0.98	0.95†	1.0	1.0	0.95	1.1†	0.98	1.0	0.96
Day 8	310.9	231.7	0.94†	0.99	1.1†	1.0	0.98	1.0	1.0	1.0	1.0	0.99	1.0	0.98
Day 9	319.8	237.0	0.87†	0.93*	1.0	0.95	0.93*	0.99	0.93†	0.94*	0.96	0.93†	0.92†	0.93*
Day 15	356.3	249.8	0.88†	0.97	1.0	0.98	0.95*	1.0	0.98	0.99	0.98[e]	0.95[e]	0.96	0.95

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body Weight Gain (%)[f]														
Day 9	21.3	11.7	6.3	5.8	2.9	4.3	13.6	9.5	-3.5	3.6	-0.77	1.5	-4.1	1.2
Day 16	37.1	16.7	15.1	10.0	11.9	11.0	25.2	17.1	6.0	10.3	NA	NA	4.8	6.4
Food Consumption[d] – Relative (g/kg of body weight/day)														
Week 1	95.0	98.3	0.94*	0.97	0.83†	0.93*	0.96	0.98	0.77†	0.87†	0.81†	0.90†	0.78†	0.86†
Week 2	89.4	94.3	0.93†	0.96	0.90†	0.99	0.98	1.0	0.88†	0.98	0.86†[e]	0.98[e]	0.89†	0.98
Clinical Observations	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Local Tolerance[g]														
Day 1 – Edema														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24 Hours Postdose														
Edema, very slight	0	0	6	1	9	6	6	5	4	9	11	10	12	11
Edema, slight	0	0	2	0	0	0	2	6	0	0	0	0	0	0
48 Hours Postdose														
Edema, very slight	0	0	4	7	13	9	8	6	7	6	10	10	8	2
Edema, slight	0	0	3	5	0	0	2	6	0	0	3	1	0	0
96 Hours Postdose														
Edema, very slight	0	0	3	3	0	0	1	0	0	0	0	2	0	0
144 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 8 – Edema														
4 Hours Postdose														
Edema, very slight	0	0	0	0	0	1	0	0	0	0	0	0	0	0
24 Hours Postdose														
Edema, very slight	0	0	3	2	3	5	11	13	7	6	4	9	7	8
Edema, slight	0	0	7	9	0	1	3	1	0	0	0	0	0	0
Edema, moderate	0	0	4	3	0	0	0	0	0	0	0	0	0	0

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
48 Hours Postdose														
Edema, very slight	0	0	3	0	0	0	8	9	1	0	0	0	0	3
Edema, slight	0	0	7	7	6	8	2	0	8	5	0	1	6	6
Edema, moderate	1	1	4	7	9	7	2	1	6	10	3	4	9	6
Edema, severe	0	0	0	0	0	0	0	0	0	0	2	0	0	0
96 Hours Postdose														
Edema, very slight	0	0	8	9	4	1	4	0	0	0	0	3	0	0
Edema, slight	0	0	1	2	1	0	0	0	0	0	0	0	0	0
144 Hours Postdose														
Edema, very slight	0	0	0	0	2	0	0	0	0	0	0	0	0	0
192 Hours Postdose														
Edema, slight	0	0	0	0	0	0	0	0	0	0	1	0	0	0
240 Hours Postdose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-	NA	NA
Day 15 – Edema														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
24 Hours Postdose														
Edema, very slight	0	0	3	6	10	8	10	7	0	0	NA	NA	0	0
Edema, slight	0	0	12	9	3	7	5	8	9	12	NA	NA	1	2
Edema, moderate	0	0	0	0	0	0	0	0	6	3	NA	NA	12	12
Edema, severe	0	0	0	0	0	0	0	0	0	0	NA	NA	2	1
48 Hours Postdose														
Edema, very slight	0	0	1	1	1	1	2	3	0	0	NA	NA	0	1
Edema, slight	0	0	3	4	2	4	3	2	1	4	NA	NA	1	3
Edema, moderate	0	0	0	0	0	0	0	0	4	1	NA	NA	4	1
96 Hours Postdose														
Edema, very slight	0	0	1	2	1	0	0	0	3	2	NA	NA	3	1
Edema, slight	0	0	2	3	1	0	0	0	0	0	NA	NA	2	3
144 Hours Postdose														
Edema, very slight	0	0	1	0	1	0	0	0	3	2	NA	NA	3	2

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Edema, slight	0	0	0	0	1	0	0	0	0	0	NA	NA	2	2
192 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
240 Hours Postdose														
Edema, very slight	0	0	0	1	2	3	2	2	3	4	NA	NA	1	3
Edema, slight	0	0	0	0	0	0	0	0	1	1	NA	NA	4	2
288 Hours Postdose														
Edema, very slight	0	0	0	1	2	0	2	1	4	5	NA	NA	3	4
Edema, slight	0	0	0	0	0	0	0	0	0	0	NA	NA	2	1
336 Hours Postdose														
Edema, very slight	0	0	0	0	0	0	0	0	2	0	NA	NA	0	0
384 Hours Postdose														
Edema, very slight	0	0	0	0	0	0	0	0	2	0	NA	NA	0	0
432 Hours Postdose														
Edema, very slight	0	0	0	0	0	0	0	0	1	0	NA	NA	0	0
480 Hours Postdose														
Edema, very slight	0	0	0	0	0	0	0	0	1	0	NA	NA	0	0
528 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
Day 1 – Erythema														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48 Hours Postdose														
Erythema, very slight	0	0	0	0	0	0	0	0	0	3	0	0	0	0
96 Hours Postdose														
Erythema, very slight	0	0	9	7	1	0	0	0	0	2	3	4	0	2
144 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 8 – Erythema														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24 Hours Postdose														
Erythema, very slight	0	0	6	5	0	0	4	1	0	0	0	0	0	0

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
48 Hours Postdose														
Erythema, very slight	0	0	7	9	0	0	1	1	0	0	0	0	0	0
Erythema, well defined	0	0	2	1	0	0	0	0	0	0	0	0	0	0
96 Hours Postdose														
Erythema, very slight	0	0	3	1	0	0	0	0	0	0	0	0	0	0
144 Hours Postdose														
Erythema, severe	0	0	0	0	5	4	0	0	0	3	4	2	3	5
192 Hours Postdose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-	-	NA	NA
240 Hours Postdose														
Erythema, severe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
288 Hours Postdose														
Erythema, severe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
336 Hours Postdose														
Erythema, severe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
384 Hours Postdose														
Erythema, severe	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
432 Hours Postdose														
Erythema, very slight	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
480 Hours Postdose														
Erythema, very slight	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
528 Hours Postdose														
Erythema, very slight	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	0	NA	NA
Day 15 – Erythema														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
24 Hours Postdose														
Erythema, very slight	0	0	1	2	1	1	1	2	0	1	NA	NA	0	0
48 Hours Postdose														
Erythema, very slight	0	0	2	1	3	1	1	0	0	0	NA	NA	1	2

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
96 Hours Postdose														
Erythema, severe	0	0	5	5	0	0	0	0	0	0	NA	NA	0	0
144 Hours Postdose														
Erythema, very slight	0	0	3	1	0	0	0	0	0	0	NA	NA	0	0
Erythema, well-defined			0	3	0	0	0	0	0	0	NA	NA	0	0
192 Hours Postdose														
Erythema, very slight	0	0	0	1	0	0	0	0	0	0	NA	NA	0	0
Erythema, severe			2	2	0	0	0	0	0	0	NA	NA	0	0
240 Hours Postdose														
Erythema, severe	0	0	2	2	0	0	0	0	0	0	NA	NA	0	0
288 Hours Postdose														
Erythema, severe	0	0	2	2	0	0	0	0	0	0	NA	NA	0	0
336 Hours Postdose														
Erythema, very slight	0	0	2	0	0	0	0	0	0	0	NA	NA	0	0
Erythema, severe	0	0	0	2	0	0	0	0	0	0	NA	NA	0	0
384 Hours Postdose														
Erythema, very slight	0	0	2	0	0	0	0	0	0	0	NA	NA	0	0
Erythema, severe	0	0	0	2	0	0	0	0	0	0	NA	NA	0	0
432 Hours Postdose														
Erythema, very slight	0	0	2	0	0	0	0	0	0	0	NA	NA	0	0
Erythema, well-defined	0	0	0	2	0	0	0	0	0	0	NA	NA	0	0
480 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
Day 1 – I/H[h]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 8 – I/H														
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96 Hours Postdose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
144 Hours Postdose														

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
I/H, slight	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Day 15 – I/H[h]	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
4 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
24 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
48 Hours Postdose														
I/H, very slight	0	0	0	0	1	0	0	0	0	0	NA	NA	0	0
96 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
144 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
192 Hours Postdose														
I/H, moderate	0	0	0	0	1	0	0	0	0	0	NA	NA	0	0
240 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
288 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
336 Hours Postdose														
1/H, slight	0	0	0	0	0	0	1	0	0	0	NA	NA	0	0
384 Hours Postdose														
I/H, very slight	0	0	0	0	0	0	1	0	0	0	NA	NA	0	0
432 Hours Postdose														
I/H, very slight	0	0	0	0	0	0	1	0	0	0	NA	NA	0	0
480 Hours Postdose	-	-	-	-	-	-	-	-	-	-	NA	NA	-	-
Ophthalmology	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Auditory	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Body temperature (°C)														
Day 1														
4 Hours Postdose	37.4	37.4	37.9†	38.3†	37.7*	38.2†	37.6	37.7	38.7†	38.4†	38.3†	38.7†	38.5†	38.5†
24 Hours Postdose	37.5	38.3	38.5†	38.6	37.0	38.4	37.8	38.5	36.7†	38.3	36.6†	38.1	37.5	39.1†
Day 8														
4 Hours Postdose	37.3	37.6	37.9†	38.4†	37.6	38.0	37.5	38.2	38.0†	38.2*	38.0†	38.4†	38.1†	38.4†
24 Hours Postdose	37.3	38.4	39.0†	39.0	38.0	38.8	38.2†	38.7	39.0†	39.0	39.0†	39.0	38.9†	39.3†

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 15														
4 Hours Postdose	38.3	38.9	38.2	38.7	37.6†	38.9	37.4†	37.6†	38.7*	39.2	38.6[e]	38.7[e]	38.6	39.1
24 Hours Postdose	38.0	39.0	38.9†	39.2	38.2	39.1	38.0	39.0	39.1†	39.4	NA	NA	39.1†	39.5*
Hematology/Coagulation[i]														
Red Blood Cell (10^6/µL)														
Day 4	7.270	7.654	7.218	7.295	7.754†	7.807	7.126	7.506	7.784†	7.589	7.796†	7.576	7.848†	7.578
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	7.708	7.419	NA	NA
Day 17	7.956	7.892	7.723	7.546	7.844	7.465*	7.751	7.248†	7.511	7.145†	NA	NA	7.670	7.115†
Hemoglobin (mmol/L)														
Day 4	8.60	8.87	8.43	8.57	8.99*	9.12	8.21*	8.70	8.93*	8.62	8.95*	8.78	9.11†	8.74
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8.75	8.43	NA	NA
Day 17	9.14	9.08	8.67†	8.66	8.69†	8.38†	8.62†	8.13†	8.14†	7.85†	NA	NA	8.31†	7.93†
Hematocrit (%)														
Day 4	41.92	41.87	40.58	40.41	42.77	42.23	40.39*	41.39	42.53	40.49	42.66	40.31	42.88	40.15
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	41.46	38.86	NA	NA
Day 17	45.03	43.45	42.43†	41.74	41.10†	39.24†	42.66†	39.50†	38.79†	37.06†	NA	NA	39.65†	37.59†
Reticulocytes (10^3/µL)														
Day 4	307.0	195.7	74.9†	69.8†	116.3†	94.9†	171.1†	143.9*	112.5†	112.3†	77.1†	79.6†	85.5†	101.3†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	192.7	184.4	NA	NA
Day 17	234.6	201.0	174.8†	199.8	190.4*	225.5	188.6*	209.9	223.3	226.7	NA	NA	172.9†	198.0
Platelets (10^3/µL)														
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	708.8	570.4	NA	NA
Day 17	1089.2	1068.1	804.7†	622.9†	805.1†	698.1†	930.6	876.8*	817.2†	702.2†	NA	NA	771.4†	704.4†
White blood cells (10^3/µL)														
Day 4	9.37	8.42	11.75	12.89†	10.57	8.72	10.00	8.31	10.91	9.05	12.89†	10.03	12.83†	10.40
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	20.12	15.27	NA	NA
Day 17	9.09	7.11	16.28†	14.50†	14.76†	11.02*	14.61†	12.74†	16.56†	14.41†	NA	NA	19.88†	15.00†
Neutrophils (10^3/µL)														
Day 4	1.50	1.11	3.43†	3.84†	1.41	1.11	1.46	1.13	1.32	1.73	2.52†	2.28†	2.00	2.52†

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8.79	6.55	NA	NA
Day 17	1.46	0.95	7.74†	6.52†	5.35†	4.14†	5.89†	5.54†	7.98†	6.96†	NA	NA	10.29†	7.37†
Monocytes (10^3/µL)														
Day 4	0.29	0.19	0.41	0.44†	0.30	0.23	0.26	0.18	0.21	0.20	0.41	0.37†	0.27	0.22
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.63	0.39	NA	NA
Day 17	0.31	0.19	0.57*	0.37*	0.63†	0.44†	0.62†	0.44†	0.55*	0.40†	NA	NA	0.50	0.31
Eosinophils (10^3/µL)														
Day 4	0.121	0.134	0.121	0.175	0.119	0.104	0.124	0.158	0.119	0.107	0.097	0.137	0.110	0.162
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.094	0.092	NA	NA
Day 17	0.109	0.094	0.101	0.099	0.106	0.152*	0.231†	0.308†	0.360†	0.508†	NA	NA	0.566†	0.573†
Basophils (10^3/µL)														
Day 4	0.026	0.026	0.038	0.057†	0.047*	0.036	0.035	0.030	0.042	0.033	0.060†	0.047†	0.065†	0.043*
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.102	0.055	NA	NA
Day 17	0.030	0.019	0.063†	0.060†	0.069†	0.039*	0.060†	0.042†	0.063†	0.043†	NA	NA	0.074†	0.039*
Large unstained cells (10^3/µL)														
Day 4	0.09	0.09	0.66†	0.59†	0.22†	0.19†	0.15	0.11	0.22†	0.31†	0.41†	0.33†	0.35†	0.37†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.38	0.87	NA	NA
Day 17	0.09	0.08	1.17†	0.86†	0.49†	0.48†	0.24†	0.43†	0.59†	0.63†	NA	NA	0.69†	0.54†
Fibrinogen (mg/dL)														
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	298.2	267.3	NA	NA
Day 17	106.1	114.4	309.1†	314.0†	271.0†	279.8*	271.4†	281.8†	310.0†	299.1†	NA	NA	323.9†	297.8†
Clinical Chemistry[i]														
Albumin (g/L)														
Day 4	29.48	31.61	26.70†	27.15†	27.48†	28.03†	28.27†	28.97†	27.41†	28.21†	27.22†	27.92†	26.79†	27.62†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	27.32	27.35	NA	NA
Day 17	28.34	30.36	26.78†	27.68†	26.67†	27.69†	27.23†	27.38†	27.26†	27.17†	NA	NA	26.68†	27.03†
Globulin (g/L)														
Day 4	27.12	27.69	29.70†	28.95	27.62	25.67	31.43†	30.33*	29.59†	29.89	28.88*	27.28	29.11*	28.68

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	28.08	26.15	NA	NA
Day 17	25.36	25.54	27.82†	29.02†	27.03	28.51	30.07†	30.12†	32.04†	29.23†	NA	NA	31.22†	30.07†
Albumin/Globulin Ratio														
Day 4	1.087	1.144	0.901†	0.938†	0.996†	1.095	0.902†	0.958†	0.929†	0.950†	0.944†	1.028†	0.923†	0.964†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.975	1.049	NA	NA
Day 17	1.119	1.192	0.963†	0.962†	0.988†	1.076	0.908†	0.910†	0.853†	0.933†	NA	NA	0.856†	0.901†
Gamma glutamyl transferase (U/L)														
Day 4	0.95	0.88	4.21†	3.67†	2.93†	2.75†	2.52†	2.32	3.32†	3.72†	3.60†	3.77†	3.25†	4.01†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3.98	4.26	NA	NA
Day 17	1.62	1.21	4.43†	3.97†	3.04†	3.32†	3.59†	3.94†	4.18†	4.40†	NA	NA	4.83†	5.05†
Acute Phase Proteins														
α–1-Acid Glycoprotein (µg/mL)														
Day 4	64.7	79.8	465.0†	401.4†	304.7†	323.6†	381.9†	378.9†	454.9†	445.0†	431.1†	390.6†	446.8†	445.6†
Day 10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	416.3	409.7	NA	NA
Day 17	50.3	52.0	429.6†	467.7†	737.0†	649.4†	437.6†	463.0†	970.9†	980.9†	NA	NA	1043.6†	826.1†
α–2-Macroglobulin (µg/mL)														
Day 4	39.8	18.1	727.0†	126.2†	223.0†	57.1†	1434.6†	330.4†	2143.1†	1639.4†	685.5†	169.6†	2159.0 †	1362.6 †
Day 17	21.2	16.1	551.7†	269.3†	394.3†	102.5†	930.4†	724.0†	5927.3 †	2692.2 †	NA	NA	4604.5 †	1937.5 †
Urinalysis (Day 17)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Immunogenicity	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Cytokines[j]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organ Weights[d]														
Spleen														
Absolute (g)	0.838	0.595	1.2	1.6†	1.3†	1.2	1.1	1.3*	1.2†	1.6†	1.2	1.3	1.3†	1.6†

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Relative (g/1000 g body weight)	2.568	2.701	1.4†	1.7†	1.3†	1.3	1.2†	1.2	1.3†	1.5†	1.5	1.4	1.4†	1.6†
Gross Pathology														
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Injection site														
Indurated[k]	0	0	10	10	7	7	7	6	6	6	10	10	7	9
Incrusted	0	0	2	2	1	0	0	0	0	0	1	1	0	0
Lymph node, iliac														
Enlarged	0	0	1	1	4	3	6	4	7	8	1	2	5	6
Spleen														
Enlarged	0	0	2	4	5	2	1	1	5	7	5	1	2	7
Histopathology (Day 17[l])														
Number Examined	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Injection site[b]														
Fibrosis intramuscular/ Interstitial														
Minimal	0	0	1	0	0	0	0	0	1	1	0	0	0	0
Mild	0	0	8	10	10	10	9	10	8	9	8	10	10	10
Moderate	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fibrosis inter-/perimuscular														
Mild	0	0	10	10	10	10	9	10	10	10	8	10	10	10
Moderate	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Inflammation, mixed, subcutis (Injection site 1)														
Mild	0	0	0	0	0	0	1	0	2	0	0	0	0	0
Moderate	0	0	9	10	10	10	7	10	8	10	9	10	9	10
Marked	0	0	0	0	0	0	2	0	0	0	0	0	1	0

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Inflammation, mixed, intramuscular/ interstitial (Injection site 1)														
Minimal	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Mild	0	0	4	8	8	4	1	4	3	8	4	3	4	9
Moderate	0	0	4	2	2	6	9	5	1	0	0	0	1	0
Inflammation, mixed, intramuscular/interstitial, multifocal (Injection site 1)														
Moderate	0	0	0	0	0	0	0	1	4	2	5	7	5	1
Inflammation, mixed, inter-/perimuscular (Injection site 1)														
Minimal	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Mild	0	0	3	0	0	0	1	0	0	0	0	0	0	0
Moderate	0	0	7	10	10	7	6	9	8	10	9	8	9	10
Marked	0	0	0	0	0	3	3	1	0	0	0	2	1	0
Inflammation, mixed, subcutis (Injection site 2)														
Mild	0	0	0[m]	0[m]	0	0	0	0	1	1	0	0	0	2
Moderate	0	0	3[m]	0[m]	0	0	0	0	9	9	0	0	10	8
Inflammation, mixed, intramuscular/interstitial (Injection site 2)														
Mild	0	0	2[m]	0[m]	0	0	0	0	5	6	0	0	4	9
Moderate	0	0	1[m]	0[m]	0	0	0	0	0	0	0	0	0	0

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Inflammation, mixed, intramuscular/interstitial, multifocal (Injection site 2)														
Minimal	0	0	1[m]	0[m]	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	0[m]	0[m]	0	0	0	0	5	4	0	0	6	1
Inflammation, mixed, inter-/perimuscular (Injection site 2)														
Minimal	0	0	1[m]	0[m]	0	0	0	0	0	0	0	0	0	0
Moderate	0	0	2[m]	0[m]	0	0	0	0	10	10	0	0	10	10
Myofiber degeneration														
Minimal	0	0	2	0	0	0	1	2	1	2	0	0	0	0
Mild	0	0	7	9	9	9	8	8	9	8	7	4	10	10
Moderate	0	0	0	0	0	0	0	0	0	0	1	5	0	0
Edema, subcutis														
Mild	0	0	1	0	1	1	4	4	1	2	0	0	1	2
Moderate	0	0	5	10	9	6	4	6	7	8	6	5	7	7
Marked	0	0	0	0	0	2	1	0	0	0	3	5	2	1
Oedema intramuscular/ Interstitial														
Minimal	0	0	1	8	6	1	2	2	1	1	1	1	0	0
Mild	0	0	1	2	1	7	6	7	7	9	8	9	10	10
Oedema inter-/ perimuscular														
Minimal	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Mild	0	0	2	1	5	0	3	1	0	2	2	1	0	0
Moderate	0	0	4	9	5	8	6	8	8	6	6	8	6	6
Marked	0	0	1	0	0	1	1	1	0	2	2	1	4	5

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hyperplasia, epidermis, widespread														
Mild	0	0	0	0	2	3	5	7	3	1	0	0	2	1
Moderate	0	0	4	9	7	4	4	1	7	9	9	10	7	9
Sciatic nerve, perineural														
Inflammation														
Minimal	0	0	0	0	0	1	0	1	3	1	0	0	1	1
Mild	0	0	0	0	0	0	0	3	2	2	0	0	2	3
Moderate	0	0	2	0	0	0	1	0	2	7	0	0	5	5
Marked	0	0	1	0	0	0	0	0	0	0	0	0	2	1
Bone femur														
Inflammation														
Minimal	0	0	0	1	0	1	0	0	1	2	0	0	0	0
Mild	0	0	0	0	0	0	0	0	2	3	0	0	2	7
Moderate	0	0	0	0	0	0	0	0	1	1	0	0	0	2
Mammary gland														
Inflammation, mixed; interstitium; focal														
Mild	0	0	0	0	0	2	0	0	1	0	2	1	0	0
Moderate	0	0	0	0	0	0	0	0	1	0	1	2	0	0
Lymph node iliac														
Plasmacytosis														
Minimal	0	0	2	2	1	1	0	0	1	0	4	1	0	0
Mild	0	0	3	1	6	6	2	1	4	4	1	6	9	2
Moderate	0	0	0	0	0	0	7	7	2	5	1	0	1	8
Marked	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Inflammation														
Minimal	0	0	4	1	0	1	0	0	0	2	1	2	1	1
Mild	0	0	1	5	0	2	0	0	3	3	1	3	7	5

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Moderate	0	0	0	0	0	0	0	0	2	2	2	2	1	0
Increased cellularity, germinal center														
Minimal	4	3	3	1	0	1	1	2	1	0	0	0	0	0
Mild	4	0	5	6	6	7	9	4	7	6	8	10	8	6
Moderate	0	0	1	1	3	2	0	2	2	3	2	0	2	4
Skeletal muscle														
Infiltration, mixed (focal, multifocal)														
Minimal	0	0	0	1	0	0	0	0	1	2	0	0	5	0
Spleen														
Increased haematopoiesis														
Minimal	0	0	0	0	3	2	0	0	0	4	0	0	2	6
Mild	0	0	0	0	0	0	0	0	2	3	0	0	0	2
Liver														
Vacuolation, hepatocellular, periportal														
Minimal	0	0	1	4	1	5	0	2	5	1	1	6	5	2
Mild	0	0	0	6	0	1	0	8	3	9	0	4	4	8
Postdose Evaluation														
Number Evaluated	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Body Temperature (°C)														
Day 36	36.8	38.3	37.4	38.8	37.6	38.8	38.2*	38.7	37.5	39.1	NA	NA	37.0	39.2
Histopathology[n]														
Number Examined	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Injection site														

2.6.7.7A Repeat-Dose Toxicity

Study Number: 38166 (continued)

Dose (µg RNA/animal)	Control (0)		BNT162a1 (30)		BNT162a1 (10)		BNT162b1 (30)		BNT162b1 (100)		BNT162c1 (30)		BNT162b2 (V8) (100)	
Dosing Frequency	QWx3		QWx3		QWx3		QWx3		QWx3		QWx2		QWx3	
Administration Sites/Dosing Day	2		1		1		1		2		1		2	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Fibrosis intramuscular/ interstitial														
Minimal	0	0	1	3	3	1	4	4	4	1	0	0	4	4
Fibrosis inter /perimuscular														
Minimal	0	0	1	1	1	4	0	1	2	5	1	4	1	0
Mild	0	0	4	4	4	1	5	4	3	0	0	0	4	4
Inflammation, inter-/ perimuscular														
Minimal	0	0	3	2	4	3	1	1	3	2	1	3	1	0
Mild	0	0	2	2	0	0	4	4	2	2	0	0	4	4
Lymph node iliac														
Plasmacytosis														
Minimal	0	0	0	2	0	2	2	3	2	2	0	1	1	1
Mild	0	0	0	1	0	1	0	0	1	3	0	0	0	3
Increased cellularity, germinal center														
Minimal	1	2	3	2	1	1	3	1	1	0	4	3	1	0
Mild	4	2	1	3	4	3	1	4	4	3	1	1	4	3
Moderate	0	0	1	0	0	1	1	0	0	2	0	0	0	1
Skeletal muscle														
Infiltration, lymphocytic														
Minimal	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Liver														
Vacuolation, hepatocellular, periportal														
Minimal	1	0	0	0	0	0	0	0	0	0	0	1	0	0

2.6.7.7A Repeat-Dose Toxicity **Study Number: 38166 (continued)**

∗ p≤0.05; † p ≤0.01, significantly different from control based on appropriate trend or pairwise comparison. A full description of the statistical decision tree can be found in the final report for this study.
- = No noteworthy findings; F = Female; GLP = Good Laboratory Practice; IFN = Interferon; I/H = Induration/Hardening; IL = Interleukin; LNP = Lipid Nanoparticle; M = Male; modRNA = Nucleoside-modified mRNA; NA = Not applicable, Not available; Neg = Negative; Pos = Positive; QW = Once weekly; RBD = Receptor binding domain; saRNA = Self-amplifying mRNA; SP2 = Spike protein P2 mutant; TNF = Tumor necrosis factor; uRNA = Uridine mRNA.

a. Final, audited study report.
b. Groups 1, 5, and 7 each received 100 µL/administration site at 2 sites for a total dose volume of 200 µL. The remaining groups each received an administration at only 1 site for a total dose volume of 60 µL (Groups 2 and 4), 20 µL (Group 3), and 70 µL (Group 6).
c. Ten (10) animals/sex/group for the dosing phase (main study animals), and 5 animals/sex/group for the recovery phase. Additional satellite animals (3/sex/group) were used only for blood sampling for cytokine analysis.
d. Group means are shown for controls. Changes from controls, expressed as multiples, are shown for groups administered test article. Statistical significance is based on actual data and not on the multiples.
e. Values represent data obtained from recovery animals (5/sex).
f. Percent differences from Day 1 are shown.
g. For local tolerance, when animals received the test article over 2 injection sites (Groups 1, 5, and 7), only the highest severity score from either injection site was used to calculate incidence.
h. No noteworthy findings were observed at all time points on Day 1 (4, 24, 48, 96, and 144 hours postdose) and Day 15 (4 and 24 hours post dose).
i. Day 4 values represent data obtained from the first 5 main study animals/sex/group and all recovery animals (5/sex/group). Day 10 and 17 values represent data obtained at the end of the dosing phase from the main study animals only (10/sex/group).
j. Data obtained from all satellite animals (3/sex/group). Cytokine parameters evaluated were IFN-γ, TNF-α, IL-1-β, IL-6, and IL-10.
k. Observation of "indurated" includes of observations of thickened injection site and/or muscle.
l. Day 10 for Group 6.
m. On Day 15, 6 animals (males 32, 34, 37, 39 and 42; female 60) were administered their third dose of BNT162a1 (Group 2) in the contralateral limb (Site II) due to local tolerance findings at the original injection site (Site I).
n. Day 31 for group 6; Day 38 for all other groups.

2.6.7.7B Repeat-Dose Toxicity

Report Title: 17-Day Intramuscular Toxicity Study of BNT162b2 (V9) and BNT162b3c in Wistar Han Rats With a 3-Week Recovery

Test Article: BNT162b2

Species/Strain: Rat/Wistar Han
Age at First Dose: 9 Weeks

Duration of Dosing: 17 Days (Dose days 1, 8, 15)
Duration of Postdose: 3 Weeks

Study Number: 20GR142
Lot Numbers: COVVAC/270320 (BNT162b2 [V9]), BCV/040620 (BNT162b3c)

Date of First Dose: 06 July 2020

Method of Administration: Intramuscular injection, QD, 60 µL/injection[a]

GLP Compliance: Yes

Vehicle/Formulation: 0.9% sterile saline/Suspension
Special Features: None
No Observed Adverse Effect Level: NA

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Number of Animals[b]	15	15	15	15	15	15
Noteworthy Findings						
Died or Euthanized Moribund	0	0	0	0	0	0
Body Weight (g)[c]						
Prior to Initiation of Dosing (Day 6)	225.28	-	-	-	1.0	-
Day 11	295.83	-	-	-	0.93†	-
Day 15	311.47	-	-	-	0.94*	-
Body Weight Change (g)						
Days 1-4	-12.64	-11.61	-19.57†	-14.21	-20.92†	-15.75
Days 4-8	+28.44	+23.34	+36.01	+25.19	+33.75	+21.98
Days 8-11	+15.23	+3.71	+0.10†	+1.37	-1.71†	+3.92
Days 11-15	+15.64	+4.06	+18.82*	+10.14†	+18.71	+11.09†
Days 1-15	+46.67	+19.50	+35.35†	+22.49	+29.83†	+21.25
Food Consumption (g)[c]						
Days 1-4	50.88	37.79	0.84†	0.87†	0.76†	0.92†
Days 4-8	90.87	74.46	1.06	0.95	1.01	0.96
Days 8-11	64.77	48.27	0.83†	0.87†	0.78†	0.84†
Days 11-15	89.35	65.27	1.03	1.02	0.99	1.05
Days 1-15	295.87	225.80	0.97	0.94	0.91*	0.95

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Clinical Observations	-	-	-	-	-	-
Local Tolerance[d]						
Day 1 – Edema						
Predose	-	-	-	-	-	-
4 Hours Postdose						
Edema, very slight	0	0	0	0	0	1
24 Hours Postdose						
Edema, very slight	0	0	5	5	7	8
Edema, slight	0	0	6	10	6	7
48 Hours Postdose						
Edema, very slight	NA	NA	1 (6)	0 (10)	0 (6)	0 (7)
Edema, slight	NA	NA	5 (6)	10 (10)	6 (6)	7 (7)
72 Hours Postdose						
Edema, very slight	NA	NA	5 (6)	0 (10)	2 (6)	0 (7)
Edema, slight	NA	NA	0 (6)	10 (10)	4 (6)	7 (7)
120 Hours Postdose						
Edema, slight	NA	NA	NA	10 (10)	4 (4)	7 (7)
144 Hours Postdose						
Edema, very slight	NA	NA	NA	0 (10)	4 (4)	0 (7)
Edema, slight	NA	NA	NA	10 (10)	0 (4)	7 (7)
Day 8 – Edema						
Predose	-	-	-	-	-	-
4 Hours Postdose	-	-	-	-	-	-
24 Hours Postdose						
Edema, slight	0	0	6	6	4	6
Edema, moderate	0	0	7	9	11	9
48 Hours Postdose						
Edema, slight	NA	NA	6 (13)	6	3	4
Edema, moderate	NA	NA	7 (13)	9	12	11
72 Hours Postdose						
Edema, very slight	NA	NA	2 (13)	1	2	0
Edema, slight	NA	NA	11 (13)	8	13	8

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Edema, moderate	NA	NA	0 (13)	6	0	7
120 Hours Postdose						
Edema, very slight	NA	NA	11 (11)	14 (14)	13 (13)	8
Edema, slight	NA	NA	0 (11)	0 (14)	0 (13)	3
144 Hours Postdose						
Edema, very slight	NA	NA	1 (11)	0 (14)	0 (13)	1
Day 15 – Edema						
Predose	-	-	-	-	-	-
4 Hours Postdose						
Edema, very slight	0	0	2	0	5	0
24 Hours Postdose						
Edema, very slight	0	0	1	0	1	0
Edema, slight	0	0	11	6	11	4
Edema, moderate	0	0	2	9	3	11
48 Hours Postdose						
Edema, very slight	NA	NA	0 (13)	1	0 (14)	0
Edema, slight	NA	NA	11 (13)	8	10 (14)	6
Edema, moderate	NA	NA	2 (13)	6	4 (14)	9
72 Hours Postdose						
Edema, very slight	NA	NA	2 (4)	0 (5)	1 (5)	0 (5)
Edema, slight	NA	NA	2 (4)	3 (5)	4 (5)	2 (5)
Edema, moderate	NA	NA	0 (4)	2 (5)	0 (5)	3 (5)
Day 1 – Erythema						
Predose	-	-	-	-	-	-
4 Hours Postdose	-	-	-	-	-	-
24 Hours Postdose						
Erythema, very slight	0	0	1	11	1	15
48 Hours Postdose						
Erythema, very slight	NA	NA	0 (6)	10 (10)	1 (6)	7 (7)
72 Hours Postdose						
Erythema, very slight	NA	NA	0 (6)	9 (10)	0 (6)	7 (7)
120 Hours Postdose						
Erythema, very slight	NA	NA	NA	9 (10)	0 (4)	7 (7)

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
144 Hours Postdose						
Erythema, very slight	NA	NA	NA	9 (10)	0 (4)	7 (7)
Day 8 – Erythema						
Predose	-	-	-	-	-	-
4 Hours Postdose	-	-	-	-	-	-
24 Hours Postdose						
Erythema, very slight	0	0	7	15	12	15
48 Hours Postdose						
Erythema, very slight	NA	NA	7 (13)	15	14	15
72 Hours Postdose						
Erythema, very slight	NA	NA	5 (13)	12	10	14
120 Hours Postdose						
Erythema, very slight	NA	NA	2 (11)	2 (14)	0 (13)	8
144 Hours Postdose	NA	NA	- (11)	- (14)	- (13)	-
Day 15 – Erythema						
Predose	-	-	-	-	-	-
4 Hours Postdose						
Erythema, very slight	0	0	0	0	1	0
24 Hours Postdose						
Erythema, very slight	0	0	0	12	0	15
48 Hours Postdose						
Erythema, very slight	NA	NA	0 (13)	3	3 (14)	12
72 Hours Postdose						
Erythema, very slight	NA	NA	0 (4)	2 (5)	0 (5)	4 (5)
Ophthalmology	-	-	-	-	-	-
Body temperature (°C)[e]						
Day 1	38.31	38.08	38.85†	38.50*	39.02†	38.58†
Day 8	37.07	37.81	38.05†	38.47†	38.33†	38.73†
Day 15	37.34	38.02	38.37†	38.15	38.43†	38.35
Hematology/Coagulation[f]						
Red Blood Cells (10^6/µL)						
Day 4	8.117	7.903	7.774*	7.381*	7.596†	7.470*
Day 17	7.584	7.423	7.169	6.872†	7.113	6.836†

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Hemoglobin (g/dL)						
Day 4	15.01	14.53	14.16*	13.56*	14.01†	13.56*
Day 17	13.82	13.83	12.53†	12.38†	12.81†	12.24†
Hematocrit (%)						
Day 4	48.04	44.91	43.37†	41.79*	43.79†	41.81*
Day 17	42.61	41.67	38.40†	38.09†	39.29*	37.21†
Mean Cell Hemoglobin (pg)						
Day 4	18.51	18.37	18.20	18.39	18.50	18.16
Day 17	18.27	18.62	17.48†	17.99†	18.01	17.89†
Mean Cell Hemoglobin Concentration (g/dL)						
Day 4	31.24	32.34	32.64†	32.49	32.04†	32.41
Day 17	32.46	33.18	32.65	32.50†	32.61	32.84
Red Cell Distribution Width (%)						
Day 4	12.27	11.11	12.83	11.39	12.44	11.97†
Day 17	11.63	11.33	14.12†	13.34†	13.73†	13.38†
Reticulocytes (10^3/µL)						
Day 4	392.1	301.7	107.4†	129.7†	104.6†	133.6†
Day 17	178.8	168.9	185.4	222.1*	194.0	203.3
White Blood Cells (10^3/µL)						
Day 4	7.60	6.01	10.70*	7.84	9.70	8.57*
Day 17	3.84	2.16	8.83†	5.70†	8.60†	6.37†
Neutrophils (10^3/µL)						
Day 4	1.083	0.920	2.470†	2.306	2.161*	2.879†
Day 17	0.674	0.409	4.449†	2.469†	4.351†	2.879†
Monocytes (10^3/µL)						
Day 4	0.109	0.093	0.199*	0.176	0.214†	0.234†
Day 17	0.071	0.056	0.234†	0.154†	0.254†	0.176†
Eosinophils (10^3/µL)						
Day 4	0.081	0.057	0.086	0.087*	0.091	0.123†
Day 17	0.056	0.029	0.141†	0.092†	0.122†	0.097†
Basophils (10^3/µL)						
Day 4	0.016	0.009	0.030*	0.017	0.037†	0.024†
Day 17	0.003	0.001	0.017†	0.008†	0.019†	0.010†

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Large Unstained Cells (10^3/µL)						
Day 4	0.046	0.030	0.187†	0.126†	0.183†	0.133†
Day 17	0.026	0.010	0.209†	0.132†	0.323†	0.190†
Fibrinogen (mg/dL)						
Day 17	253.1	217.2	596.7†	541.9†	606.1†	563.1†
Clinical Chemistry[g]						
Albumin/Globulin Ratio						
Day 4	1.88	1.98	1.70†	1.71†	1.69†	1.69†
Day 17	1.85	1.96	1.65†	1.61†	1.65†	1.66†
Total Protein (g/dL)						
Day 4	6.10	6.26	5.90	5.65†	5.85	5.94
Day 17	5.39	5.44	5.51	4.98†	5.41	4.96†
Albumin (g/dL)						
Day 4	3.98	4.16	3.71†	3.56†	3.68†	3.73†
Day 17	3.50	3.60	3.43	3.07†	3.38	3.09†
Globulin (g/dL)						
Day 4	2.13	2.10	2.19	2.09	2.18	2.21
Day 17	1.89	1.84	2.08*	1.91	2.03	1.88
Acute Phase Proteins[g]						
α2-Macroglobulin (µg/mL)						
Day 4	113.4	212.1	2318.1†	703.8†	3911.6†	887.1†
Day 17	14.0	33.1	990.6†	521.0†	1794.2†	592.0†
α1-Acid Glycoprotein (µg/mL)						
Day 4	174.358	239.774	1642.265†	1906.314†	2351.791†	1677.103†
Day 17	47.672	95.959	1835.986†	1491.849†	2021.083†	1651.071†
Urinalysis (Day 17)	-	-	-	-	-	-
Immunogenicity	Neg	Neg	Pos	Pos	Pos	Pos
Organ Weights (Day 17)[c]						
Spleen						
Absolute (g)	0.5951	0.4382	1.29†	1.55†	1.34†	1.41†
Relative (g/100 g body weight)	0.2008	0.2202	1.42†	1.59†	1.52†	1.47†
Relative (g/g brain weight)	0.3120	0.2353	1.29†	1.62†	1.34†	1.43†

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Gross Pathology (Day 17)						
Number Examined	10	10	10	10	10	10
Injection site						
Abnormal color, pale/dark	0	1	2	3	1	0
Abnormal consistency, firm	0	0	2	4	2	7
Lymph node, draining						
Abnormal size, enlarged	0	0	1	1	0	4
Spleen						
Abnormal size, enlarged	0	0	0	0	0	1
Histopathology (Day 17)						
Number Examined[h]	10	10	10	10	10	10
Injection site						
Inflammation						
Minimal	4	5	0	0	0	0
Mild	0	0	7	7	5	9
Moderate	0	0	3	3	5	1
Edema						
Mild	0	0	8	9	8	9
Moderate	0	0	1	1	1	1
Lymph Node Iliac, Draining						
Increased cellularity, Plasma cell						
Minimal	0	0	1 (9)	1	4	1
Mild	0	0	4 (9)	1	3	5
Moderate	0	0	2 (9)	7	1	1
Increased cellularity, Germinal center						
Minimal	1	1	2 (9)	3	2	4
Mild	1	1	4 (9)	2	6	2
Lymph Node, Inguinal						
Increased cellularity, Plasma cell						
Minimal	0 (9)	0	1	2	1	4
Increased cellularity, Germinal center						
Minimal	0 (9)	1	1	3	1	6
Mild	1 (9)	0	4	3	5	3

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Liver						
Vacuolation, Hepatocyte; Periportal						
Minimal	0	0	5	10	7	7
Spleen						
Increased cellularity, hematopoietic cell						
Minimal	0	0	10	9	10	10
Increased cellularity, Germinal center						
Minimal	0	0	5	6	5	5
Bone marrow, Sternum						
Increased cellularity, hematopoietic cell						
Minimal	0	0	10	10	10	10
Postdose Evaluation						
Number of Animals	5	5	5	5	5	5
Body Weight (g)[c]						
Day 11	330.74	-	1.05	-	1.00	-
Day 15	333.60	-	1.06	-	1.00	-
Day 18	341.42	-	1.05	-	1.01	-
Day 21	347.88	-	1.06	-	1.02	-
Food Consumption (g)[c]						
Days 1-21	383.66	-	1.15	-	1.08	-
Hematology/Coagulation						
Red Cell Distribution Width (%)						
Day 22	11.93	10.80	13.48†	13.04†	13.33*	13.32†
Clinical Chemistry						
Albumin/Globulin Ratio						
Day 22	1.76	1.90	1.72	1.72*	1.70	1.80
Globulin (g/dL)						
Day 22	2.10	2.26	2.26†	2.40	2.18	2.42
Local Tolerance						
Recovery Day 1 – Edema						
72 Hours Postdose						
Edema, slight	NA	NA	2	3	4	2
Edema, moderate	NA	NA	0	2	0	3

2.6.7.7B Repeat-Dose Toxicity

Study Number: 20GR142 (continued)

Dose (µg RNA)	Saline Control (0)		BNT162b2 (V9) (30)		BNT162b3c (30)	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Immunogenicity	Neg	Neg	Pos	Pos	Pos	Pos
Gross Pathology (Day 22)						
Number Examined	5	5	5	5	5	5
Lymph Node, Draining						
Abnormal size, enlarged	0	0	1	0	0	1
Lymph Node, Inguinal						
Abnormal size, enlarged	0	0	0	0	0	1
Histopathology (Day 22)						
Number Examined[i]	5	5	5	5	5	5
Injection site						
Inflammation						
Minimal	0	0	5	5	5	5
Lymph Node, Draining						
Increased cellularity, Plasma cell						
Minimal	0 (4)	0	4	4	5	3
Increased cellularity, Germinal center						
Minimal	0 (4)	1	3	2	2	4
Mild	0 (4)	0	1	1	2	1
Infiltration, Macrophage						
Minimal	0 (4)	0	2	1	2	1
Mild	0 (4)	0	1	2	2	3
Lymph Node, Inguinal						
Increased cellularity, Plasma cell						
Minimal	0	0	0	0	0	1
Increased cellularity, Germinal center						
Minimal	2	2	3	1	2	3
Infiltration, Macrophage						
Minimal	0	0	0	0	1	1
Spleen						
Increased cellularity, Germinal center						
Minimal	0	0	1	2	1	2

2.6.7.7B Repeat-Dose Toxicity **Study Number: 20GR142 (continued)**

∗ p ≤0.05; † p ≤0.01, significantly different from control based on appropriate trend or pairwise comparison. A full description of the statistical decision tree can be found in the final report for this study.
- = No noteworthy findings; F = Female; GLP = Good Laboratory Practice; M = Male; NA = Not applicable, results not yet available; Neg = Negative; Pos = Positive; QD = Once daily.
a. Each animal received a single intramuscular injection on each dose day.
b. Ten (10) animals/sex/group for the dosing phase (main study animals), and 5 animals/sex/group for the recovery phase.
c. Group means are shown for controls. Changes from controls, expressed as multiples, are shown for groups administered test article. Statistical significance is based on actual data and not on the multiples.
d. Fifteen (15) animals/sex/group examined unless otherwise indicated in ( ).
e. Values represent the highest group mean postdose body temperature after each dose.
f. Day 4 mean values for 7 animals/sex/group.; Day 17 mean values for 9 or 10 animals/sex/group.
g. Day 4 mean values for 8 animals/sex/group.; Day 17 mean values for 9 to 10 animals/sex/group.
h. Ten (10) animals/sex/group examined unless otherwise indicated in ( ).
i. Five (5) animals/sex/group examined unless otherwise indicated in ( ).

2.6.7.12 Reproductive and Developmental Toxicity – Fertility and Development

Report Title: A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by Intramuscular Administration in the Wistar Rat

Test Article: BNT162b2

Design Similar to ICH 4.1.1, 4.1.2 and 4.1.3: Yes
Species/Strain: Rat/Wistar Han
Age at First Dose (F): 11 weeks
Date of First Dose: 27 July 2020

Special Features: None

No Observed Adverse Effect Level: Not reported

Duration of Dosing (F): 4 Days (21 and 14 days prior to mating, GD 9, GD 20)
Day of Mating (F): GD 0
Day of Cesarean Section: GD 21
Day of Dams and Pups Necropsy: PND 21

Method of Administration: Intramuscular injection, 0.06 mL/injection
Vehicle/Formulation: 0.9% sterile saline/Suspension
Control Article: Sterile physiological saline (0.9 % NaCl)

Study Number: 20256434
Sponsor Reference Number: RN9391R58
Lot Numbers: CoVVAC/100320 (BNT162b1), CoVVAC/270320 (BNT162b2), BCV/040620 (BNT162b3)
GLP Compliance: Yes

Dose (µg mRNA)[a]	Saline Control (0)	BNT162b1 (30)	BNT162b2 (30)	BNT162b3 (30)
Dams				
Number of Females				
Caesarean Subgroup	22	22	22	22
Littering Subgroup	22	22	22	22
Clinical Observations				
Injection site				
Premating swelling[b]	0	43	44	43
Gestation swelling[b]	0	5	10	20
Lactation swelling[b]	1	0	3	2
Premating Body Weight (g)[c]				
Prior to Initiation of Dosing (Day 1)	216.49	1.00	1.01	1.02
Prior to Initiation of Mating (Day 22)	240.13	0.99	1.00	1.01

2.6.7.12 Reproductive and Developmental Toxicity – Fertility and Development

Study Number: 20256434 (continued)

Dose (µg mRNA)[a]	Saline Control (0)	BNT162b1 (30)	BNT162b2 (30)	BNT162b3 (30)
Premating Body Weight Change (g)				
Days 1-4	4.85	-0.55§	-0.64§	-0.17§
Days 1-22	23.64	20.00	20.39	20.94
Gestation Body Weight (g)[c]				
End of Gestation (GD 21)	365.98	0.98	0.96*	0.96*
Gestation Body Weight Change (g)				
GD 9-12	13.55	7.48§	5.70§	5.73§
GD 18-21	34.10	29.33§	24.82§	29.24§
Lactation Body Weight	-	-	-	-
Premating Food Consumption (g)[c]				
Days 1-8	18.49	0.90§	0.91§	0.91§
Days 1-22	18.43	0.97§	0.98	0.98
Gestation Food Consumption (g)[c]				
GD 9-12	22.95	0.87§	0.84§	0.83§
GD 18-21	23.41	0.98	0.97	0.99
Lactation Food Consumption	-	-	-	-
Number of Females Paired	44	44	44	44
Number of Females Failed to Mate	0	1	0	0
Number of Females Inseminated	44	43	44	44
Number of Pregnant Females	43	41	42	44
Number of Mistimed Pregnancy Females	0	0	0	1
Number of Not Pregnant Females	1	2	2	0
Number Euthanized Moribund Post-partum (Littering subgroup)	0	0	0	1[d]
Number Total Litter Death Post-partum (Littering subgroup)	0	1	0	1
Necropsy Observations (Macroscopic)				
Injection site				
Firm area	0	7	9	14
Enlarged	0	7	8	14
Oedematous area	0	0	1	0
Pale	0	2	4	10

2.6.7.12 Reproductive and Developmental Toxicity – Fertility and Development

Study Number: 20256434 (continued)

Dose (µg mRNA)[a]	Saline Control (0)	BNT162b1 (30)	BNT162b2 (30)	BNT162b3 (30)
Cesarean Subgroup				
Cesarean Section Observations				
Number Evaluated	21	20	21	22
Mean Number Corpora Lutea	14.7	15.3	15.5	15.0
Mean Number Implantations	14.1	14.6	14.0	13.8
Mean % Preimplantation Loss	4.09	4.77	9.77*	7.96
Mean % Postimplantation Loss	6.10	3.36	5.85	8.64
Mean Number Early Resorptions	0.8	0.5	0.7	1.0
Mean Number Late Resorptions	0.1	0.0	0.2	0.2
Fetuses				
Number Fetuses /Litters Evaluated	277/21	282/20	276/21	275/22
Mean Number Live Fetuses	13.2	14.1	13.1	12.5
Mean Number Dead Fetuses	0	0	0	0
Mean Fetal Body Weight (both sex) (g)	4.89	4.86	4.90	4.84
Sex Ratios (% males)	46.96	48.09	50.66	49.84
Fetal Observations				
External Malformations	-	-	-	-
External Variations/Abnormalities	-	-	-	-
Number Fetuses/Litters Evaluated	277/21	282/20	276/21	275/22
Visceral Malformations	-	-	-	-
Visceral Variations/Abnormalities	-	-	-	-
Number Fetuses/Litters Evaluated	133/21	135/20	132/21	132/22
Skeletal Malformations	-	-	-	-
Skeletal Variations/Abnormalities	-	-	-	-
Number Fetuses/Litters Evaluated	144/21	147/20	144/21	143/22
Littering Subgroup				
Number with Mistimed Pregnancy	0	0	0	1
Number with Total Litter Death	0	1	0	1
No. of Natural Deliveries	22	21	21	20
Number Euthanized Moribund Post-Partum	0	0	0	1[d]
No. of Litters with Stillborn Pups	3	4	2	2
No. of litters with All Stillborn Pups	0	0	0	1

2.6.7.12 Reproductive and Developmental Toxicity – Fertility and Development

Study Number: 20256434 (continued)

Dose (µg mRNA)[a]	Saline Control (0)	BNT162b1 (30)	BNT162b2 (30)	BNT162b3 (30)
Mean No. Pups/Litter	13.3	11.9	13.1	11.4*
Mean No. Liveborn Pups	13.0	11.0	13.0	11.3*
No. of Total Litters Losses	0	1	0	1
Pre-Birth Loss (%)	6.80	12.22	8.22	13.76*
No. of viable litters at Weaning (PND 21)	22	20	21	19
Gestation Index (%)	100	100	100	95
Live Birth Index (%)	98.0	93.2	99.3	94.7
Postnatal Survival to Day 4 (%)	99.0	98.3	98.9	99.1
Postnatal Survival to Weaning (No. of pups)	175	154	163	152
Lactation Index (PND 4-PND 21) (%)	99.4	100.0	100.0	100.0
Sex ratio at Weaning (PND 21) (Males %)	49.7	50.6	47.6	49.3
Change in Pup Body Weight (g)	-	-	-	-
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	-
Immunogenicity				
Dams	Neg	Pos	Pos	Pos
Fetuses	Neg	Pos	Pos	Pos
Pups	Neg	Pos	Pos	Pos

* $p<0.05$, § $p<0.001$; Dunnett Non-Parametric 2-Sided. A full description of the statistical decision tree can be found in the final report for this study.
- = No noteworthy findings; F = female; GD = gestation day; GLP = Good Laboratory Practice; ICH = International Conference on Harmonisation; mRNA = messenger RNA; Neg = negative; PND = postnatal day; Pos = positive.
a. Each dose consisted of a 0.06 mL intramuscular injection in alternating quadriceps muscles.
b. Complete recovery was noted between each of the dose administrations. Swelling (associated or not with limping and/or piloerection for 1 or 2 days after the second dose only) was noted at the injection site.
c. Group means are shown for controls. Changes from controls, expressed as multiples, are shown for groups administered test article. Statistical significance is based on actual data and not on the multiples.
d. Difficulties during parturition.

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R&D STUDY REPORT No. R-20-0085

COVID-19: IMMUNOGENICITY STUDY OF THE LNP-FORMULATED MODRNA ENCODING THE VIRAL S PROTEIN-V9

Version 04
Date: 23 NOV 2020

Reported by (b) (6)

Test item: BNT162b2 (animal trial material)
Key words: Coronavirus, COVID-19, modRNA, ATM, mouse, immunogenicity

This R&D report consists of 93 pages.

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LIST OF ABBREVIATIONS

AH-1	Irrelevant peptide derived from endogenous retroviral gene product envelope glycoprotein 70
ATM	Animal trial material
BCS	Body Conditioning Score
BNT162	BioNTech's SARS-CoV-2 vaccine candidate
CD	Cluster of differentiation
ConA	Concanavalin A
COVID-19	Coronavirus disease emerged 2019
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immune absorbent spot
FBS	Fetal bovine serum
GFP	Green fluorescent protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
Hsopt10	Nucleoside optimization protocol 10 based on *Homo sapiens* databank
ICS	Intracellular cytokine staining
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
i.m.	Intramuscularly
K_D	Binding affinity
k_{off}	Dissociation rate constant (off-rate)
k_{on}	Association rate constant (on-rate)
LNP	Lipid nanoparticle
LLOQ	Lower limit of quantification
MACS	Magnetic cell separation
modRNA	Nucleoside-modified mRNA
nAb	Neutralizing antibody
No.	Number
OD	Optical density
PBS	Phosphate-buffered saline
PMA	Phorbol 12-myristate 13-acetate
pVNT	Pseudovirus-based neutralization test
RBD	Receptor-binding domain
RNA	Ribonucleic acid
S protein	Spike protein
S1	Subdomain 1 of the S protein
S2	Subdomain 2 of the S protein
saRNA	Self-amplifying mRNA
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SPR	Surface plasmon resonance
T_H1/T_H2	Type 1/2 helper T cells
TNF	Tumor necrosis factor
ULOQ	Upper limit of quantification
uRNA	Uridine-containing mRNA
V	Variant
VSV	Vesicular stomatitis virus

RESPONSIBILITIES

Person responsible for the study:	(b) (6) (b) (6) BioNTech RNA Pharmaceuticals GmbH	23NOV20 Date
Author:	(b) (6) BioNTech SE	Date
Reviewer:	(b) (6) (b) (6), BioNTech RNA Pharmaceuticals GmbH	23 NOV 20 Date
QA representative:	(b) (6) BioNTech SE	23NOV20 Date

Meaning of the signatures:

Person responsible for the study: I am responsible for the content of the R&D report and confirm that it represents an accurate record of the results. This study was performed according to the SOPs and methods as well as the rules and regulations described in the report.

Author: I am the author of this document.

Reviewer: I reviewed the R&D report and confirm that this document complies with the scientific and technical standards and requirements.

QA representative: I confirm that this document complies with the relevant quality assurance requirements.

The author gave approval to this document via e-mail according to CC-20-0081 (see attachment

1 SUMMARY

BioNTech is developing RNA-based vaccines designed to protect against the novel coronavirus disease that emerged in 2019 (COVID-19). The project involves testing three RNA platforms which are under development at BioNTech with the surface or spike protein (S protein) of the novel coronavirus (SARS-CoV-2) as the viral antigen.

In the present study, the immunogenicity of a nucleoside-modified mRNA (modRNA) encoding the antigen variant 9 (V9) of the generated variants of the S protein, BNT162b2, was investigated. Four groups of eight female BALB/c mice were immunized on day 0 with doses of 0.2 µg, 1 µg, or 5 µg per animal of the modRNA encapsulated in lipid nanoparticles (LNPs), or with the buffer alone (control group), by intramuscular injection. Blood was collected on days 7, 14, 21, and 28 after immunization to analyze the antibody immune response by ELISA and pseudovirus-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) and were performed to assess cytokine responses.

The vaccine candidate was highly immunogenic; treatment with all tested BNT162b2 doses induced a strong immune response across the observation period of 28 days. Total IgG ELISA showed that the construct induced a strong, dose-dependent generation of antibodies against the S1 antigen and the receptor-binding domain (RBD). Vaccine-elicited IgG had a strong binding affinity for S1 and the RBD, both had low off-rates, as detected by surface plasmon resonance spectroscopy (SPR). In pVNT analysis, all mice developed functional neutralizing antibodies starting at 14 days after immunization and increasing up to final study day. The summary of antibody titers on day 28 is as follows:

	BNT162b2 0.2 µg	BNT162b2 1 µg	BNT162b2 5 µg
Anti-S1 protein total IgG [µg/mL]	73.0 ± 10.4	205.9 ± 21.0	392.7 ± 28.9
Anti-RBD protein total IgG [µg/mL]	83.1 ± 12.3	241.7 ± 17.2	448.6 ± 28.6
pVN_{50} titer [reciprocal dilution]	33.0 ± 9.8	192.0 ± 31.4	312.0 ±35.1

By profiling the IgG subtypes, a balanced IgG2a/IgG1 response was detected for the higher doses, while the low dose induced a response with higher IgG1 than IgG2 levels. Cellular assays and cytokine profiling revealed that in addition to a cytotoxic $CD8^+$ T-cell response, a proinflammatory, T_H1-specific response was activated after peptide stimulation. Therefore, BNT162b2 is a promising candidate for further testing in clinical trial.

(b) (6)

BioNTech RNA Pharmaceuticals GmbH

23NOV20

Date

2 GENERAL INFORMATION

Sponsor

BioNTech RNA Pharmaceuticals GmbH
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55131 Mainz
Germany

Test Facility

BioNTech SE
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2.1 Participating Personnel

Responsible person: (as defined in SOP-100-024)	(b) (6) An der Goldgrube 12 55131 Mainz
Author:	(b) (6) BioNTech SE
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
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Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter:	(b) (6) BioNTech Diagnostics GmbH
Experimenter:	(b) (6) BioNTech Diagnostics GmbH
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH

2.2 Study Dates

Start of experiments: 31 MAR 2020

Completion of experiments: 17 SEP 2020

2.3 Guidelines and Regulations

All experiments are executed in accordance with the existing standard operating procedures and described processes from BioNTech SE. Applicable documents are listed below.

- Animal test application approval number: G18-12-100, amendment from 18.02.2020 (approved 20 FEB 2020)
- SOP-010-017 Brutschränke - Biolytics
- SOP-010-028 Vi-Cell XR
- SOP-010-045 Brutschrank HERAcell 150i
- SOP-010-047 Zentrifuge Eppendorf 5810/5810R
- SOP-010-051 Tiefkühlschränke -80 °C
- SOP-010-058 Sicherheitswerkbank Klasse II
- SOP-010-086 Zentrifuge Thermo Scientific Heraeus Pico und Fresco 17
- SOP-010-099 CTL ELISPOT Reader
- SOP-020-009 Ansetzen von Medien und Zusätzen für die Zellkultur
- SOP-030-043 Kryokonservierung von Zellen
- SOP-030-071 Abtöten von Mäusen
- SOP-030-072 Fixiergriff und Ohrmarkierung bei Mäusen
- SOP-030-073 Betäubung bei Mäusen
- SOP-030-074 Blutentnahme bei Mäusen
- SOP-030-078 Isolierung muriner Splenozyten
- SOP-030-079 Intramuskuläre Applikation bei Mäusen
- SOP-030-110 IFNγ ELISpot (murin)
- SOP-030-112 Durchführung eines virusprotein-spezifischen ELISA
- SOP-090-013 Biological safety in laboratories
- SOP-110-022 Entsorgung von Biostoffabfällen

2.4 Changes and Deviations

This R&D study was conducted according to R&D plan P-20-0085.

A change occurred in the pVNT. It was planned to perform this analysis with an external partner, (b) (4) . However, the CRO had no pVNT or VNT in place when samples were ready to analyze. Therefore, an internal assay was developed using the VSV-based pseudovirus to analyze for neutralizing antibodies.

Furthermore vaccine-induced SARS-CoV-2 specific antibodies were analyzed for their affinity toward recombinant SARS-CoV-2 S and RBD protein via surface plasmon resonance (SPR) spectroscopy. Affinity measurements were only conducted with day 28 sera of the 5 µg BNT162b2 dose cohort.

Another change occurred in the protocol for murine ELISpot, described within SOP-030-110. The described change resulted in faster dryness of the ELISpot plate and thus its readiness for the subsequent protocol step; analysis of spot numbers per well via ImmunoSpot® S6 Core Analyzer, CTL. This change has no impact on performance of the protocol.

Furthermore, in a first run with fresh splenocytes a miscalculation of cells in the group immunized with 5 µg modRNA occurred. Therefore, a second ELISpot run was included with frozen splenocytes.

Because the utilized major histocompatibility complex (MHC) I/II blockade was not effective in determining T-cell subtypes, an additional ELISpot analysis was performed after separation of $CD4^+$ and $CD8^+$ cells by MACS isolation to identify the responding T-cell subtype (group 4 only).

Cytokine concentrations in supernatants of re-stimulated splenocytes were determined using a bead-based, T_H1/T_H2 mouse ProcartaPlex immunoassay. An intracellular cytokine staining was added for T_H1/T_H2 cytokine analysis.

2.5 Documentation and Archive

Study plans and reports are stored and archived according to SOP-100-003 Archiving of Paper-Based Documents.

Raw data and evaluated data are saved at:

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\00_Pharmacology\ mCorVAC#11_modRNA-V9
- Animal Models & Facility: Lab book No. 1893
- Infectious Disease Vaccines (ELISA): Lab book No. 1858, 1978
- Infectious Disease Vaccines (ICS): Lab book No. 1937
- Immunomodulators: Lab book No. 1935, 1936
- Cancer Vaccines: Lab book No. 1934
- New Scaffolds: Lab book No. 2009

3 INTRODUCTION

3.1 Background

In December 2019, an outbreak of pneumonia of unknown cause in Wuhan, Hubei province in China was reported. The disease spread rapidly and in January 2020, the agent was identified. By 21 June 2020, infection with the novel coronavirus (SARS-CoV-2) was confirmed in over 8,700,000 people with more than 460,000 casualties[1]. A vaccine is urgently needed and BioNTech decided to develop a rapid vaccine project based on the surface or spike protein (S protein) of the virus as the viral antigen. The S protein is a trimer and during viral egress, the precursor protein is cleaved in S1 and S2 (Figure 1). While the S1 domain recognizes the host receptor, the S2 domain is essential for the membrane fusion of viral envelope and endosomal membrane. To initiate the membrane fusion, the S2 domain undergoes a conformational change within the central helix domain.

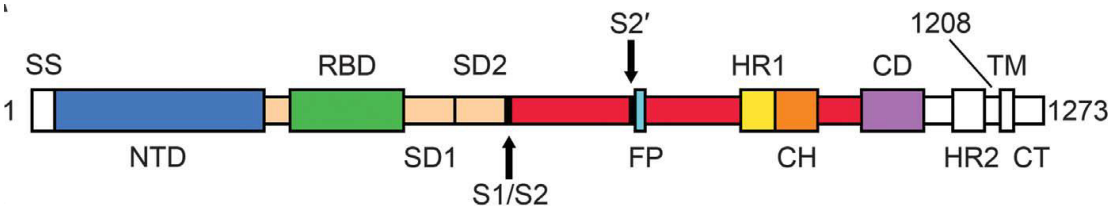

Figure 1: Schematic overview of the S protein organization of the SARS-CoV-2 S protein.

The sequence within the S1 subunit consists of the signal sequence (SS) and the receptor-binding domain (RBD) which is the key subunit within the S protein which is relevant for binding to the human cellular receptor ACE2. The S2 subunit contains the S2 protease cleavage site (S2') followed by a fusion peptide (FP) for membrane fusion, heptad repeats (HR1 and HR2) with a central helix (CH) domain, the transmembrane domain (TM) and a cytoplasmic tail (CT); source: modified from (Wrapp et al. 2020).

Based on these features, the S protein is the target of the neutralizing antibody (nAb) that binds dominantly to the RBD of the S protein. Vaccine candidates selected for non-clinical testing include the following vaccine antigens:

- A secreted variant of the RBD of the SARS-CoV-2 S protein (called V5) (Kirchdoerfer et al. 2018)
- Membrane-tethered full-length S protein with two point mutations within the central helix domain (called V8/V9). Mutation of the two amino acids to proline, (KV286-287PP) retains the S protein in an antigenically optimal prefusion conformation (called V8 or V9) (Wrapp et al. 2020, Pallesen et al. 2017)

[1] Coronavirus disease (COVID-2019) situation report 153, World Health Organization; www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

The development of *in vitro* transcribed RNA as an active platform for the use in infectious disease vaccines is based on the extensive knowledge of the company in RNA technology, which has been gained over the last decade. The core innovation is based on *in vivo* delivery of a pharmacologically optimized, antigen-coding RNA vaccine to induce robust neutralizing antibodies and accompanying/concomitant T cell response to achieve protective immunization with minimal vaccine doses (Vogel et al. 2017, Moyo et al. 2018, Pardi et al. 2017).

At BioNTech, there are three different RNA platforms under development, namely non-modified uridine-containing mRNA (uRNA), nucleoside-modified mRNA (modRNA), and self-amplifying RNA (saRNA). It is unknown today which RNA vaccine platform performs best in terms of activation and duration of a potent immune response. Therefore, BioNTech has developed a project plan that is based on testing GMP-produced, available material that has already been tested in clinical trials. The three vaccine platforms will be tested for each antigen construct in non-clinical mouse studies and tested for their virus-neutralizing response and the total amount of IgG antibodies developed against the S protein. Candidates that induce a high fraction of nAb within the total IgG population are desired. This report covers a mouse study testing modRNA encoding the antigen variant 9 (V9) of the generated variants of the S protein.

3.2 Objectives

In this study, the primary objective was to understand the immunogenicity of the designed construct. For this purpose, a dose titration in BALB/c mice was performed with the LNP-formulated modRNA encoding the antigen variant 9 (V9) of the generated variants of the S protein (V9 main characteristics: S protein full-length with two point mutations, opt1 sequence optimization that increases the GC-content of the coding sequence). The immune response was analyzed focusing on the antibody immune response and included the analysis of the IFN-γ release of splenocytes at the end of study as well as assessment of cytokine/chemokine responses.

3.3 Study Design

Four groups of eight female BALB/c mice were immunized once (on day 0) with BNT162b2 at three different doses, or with the buffer alone (control group). Immunizations were given intramuscularly (i.m.) in a dose volume of 20 µL. Blood was collected once weekly for three weeks (days 7, 14, and 21) to analyze the antibody immune response by ELISA and pseudovirus-based neutralization assay (pVNT). At the end of the study (on day 28), blood was collected for ELISA and pVNT analyses (all samples), as well as for affinity measurements of vaccine-induced antibodies toward recombinant SARS-CoV-2 S and RBD via SPR (high-dose cohort samples only). Animals were then euthanized for spleen collection and additional analysis of the T-cell response in splenocytes by ELISpot, Luminex assay, and ICS (see Table 1).

Table 1: Study design

Group no.	No. of animals	Vaccine/ batch	Concentration of active component [µg/animal]	Immunization day	Dose volume [µL] / route	Blood collection day	End of study day
1	8	Buffer	-	0	20 / i.m.	7, 14, 21, 28	28
2	8	BNT162b2 /RBP020.2	0.2	0	20 / i.m.	7, 14, 21, 28	28
3	8	BNT162b2 /RBP020.2	1	0	20 / i.m.	7, 14, 21, 28	28
4	8	BNT162b2 /RBP020.2	5	0	20 / i.m.	7, 14, 21, 28	28

4 MATERIALS AND METHODS

4.1 Test Item

BNT162b2, animal trial material (ATM): For CoAs see Appendix 2: Certificates of Analysis.

- RNA batch: RNA-RF200321-06
- Polymun batch RBP020.2 LNP with the lot: CoVVAC/270320

4.2 Control Item

- PBS+300 mM sucrose (from Polymun)

4.3 Test System

- 32 female BALB/c mice at an age of 9 weeks at study start.

4.4 Materials

Table 2: Materials

Product name	Application/ specification	Article no.	Working dilution	Provider
15 mL/50 mL tube	Conical bottom, PP, 30/115 MM, CELLSTAR®	188271/ 227261	N/A	Greiner Bio-One GmbH
2 mL tube	CRYO.S, round bottom	122278	N/A	Greiner Bio-One GmbH
2-Mercaptoethanol	50 mM	31350-010	N/A	Gibco
8-channel manifold	Polypropylene	BR704526-1EA	N/A	Sigma-Aldrich Chemie GmbH
96-well flat-bottom plate	pVNT	655160	N/A	Greiner
96-well microplate	Clear round bottom TC-treated microplate, with lid, sterile	3799	N/A	Corning Holding GmbH
96-well V-bottom plate	pVNT	651180	N/A	Greiner
AffiniPure goat anti-mouse IgG	SPR	115-005-071	N/A	Jackson ImmunoResearch
Alexa Fluor® 488 anti-mouse TNF-α antibody, clone MP6-XT22	ICS	506313	1:100	BioLegend
Amine coupling kit	SPR	BR100050	N/A	GE Healthcare
Ammonium chloride	NH_4Cl	A0988,5000	N/A	AppliChem GmbH
Anti-rat/hamster Ig, κ/negative control (FBS*)	Compensation Particles Set	552845 component no. 51-90-9000949	1 drop	BD

Product name	Application/ specification	Article no.	Working dilution	Provider
Anti-VSV-G antibody	Clone 8G5F11	EB0010	N/A	Kerafast
APC anti-mouse IL-2 antibody	ICS	503810	1:100	BioLegend
BD Pharmingen™ purified rat anti-mouse CD16/CD32	Mouse BD Fc Block™ (2.4G2)	553142	1:100	BD
Blocker™ bovine serum albumin (BSA) in PBS (10×)	ICS	7011150	1×	ThermoFisher
Brilliant Violet 510™ anti-mouse CD4 antibody	ICS	100559	1:200	BioLegend
Brilliant Violet 711™ anti-mouse IL-4 antibody	ICS	504133	1:200	BioLegend
Brilliant Violet 785™ anti-mouse CD25 antibody PC61	ICS	102051	1:200	BioLegend
BV421 rat anti-mouse CD8a antibody	ICS	100753	1:200	BioLegend
Capillary pipettes	minicaps®, blood sampling, 4 µL/10 µL, not heparinized	9000104/ 9000110	N/A	Hirschmann Laborgeräte GmbH & Co.KG
Casein blocking buffer 10×	ELISA	B6429-500ml	N/A	Sigma-Aldrich Chemie GmbH
CM5 sensor chip	SPR	BR100012	N/A	GE Healthcare
Combitips advanced®	Biopur®, 50 mL	0030089693	N/A	Eppendorf Vertrieb Deutschland GmbH
Concanavalin A	From *Canavalia ensiformis* (Jack bean, 5 mg),Type IV-S, lyophilized	C0412-5MG	N/A	Sigma-Aldrich Chemie GmbH
Cover films	ELISA	RATI6018410	N/A	VWR International GmbH
Dimethyl sulfoxide (DMSO)	For cell culture	A3672,0100	N/A	AppliChem GmbH
DPBS	No calcium, no magnesium	14190-094	1 ×	Thermo Fisher Scientific
Easystrainer 70 µm	For 50 mL tubes	542070	N/A	Greiner Bio-One GmbH
eBioscience™ Fixable Viability Dye eFluor™ 780	ICS	65-0865-18	1:1,000	ThermoFisher
Eppendorf safe-lock tubes	0.5 mL/ 1.5 mL/ 2.0 mL/ 5.0 mL, Eppendorf Quality™	0030121023/0030120086/0030120094/0030119401	N/A	Eppendorf Vertrieb Deutschland GmbH

Product name	Application/ specification	Article no.	Working dilution	Provider
Ethylenediaminetetraacetic acid solution	EDTA	03690-100ML	N/A	Sigma-Aldrich Chemie GmbH
Fetal bovine serum (FBS)	Non-USA origin, sterile-filtered	F7524	N/A	Sigma-Aldrich Chemie GmbH
Filtration unit for medium flasks	High Performance, PES, 0.45 µm, 1,000 mL	514-0301	N/A	VWR International GmbH
Goat anti-mouse IgG (POX)	Whole IgG Fc y fragment, secondary antibody, IgG isotype-specific ELISA	115-035-071	1:15,000	Jackson ImmunoResearch via Dianova
Goat anti-mouse IgG HRP	ELISA	115-035-071	1:15,000	Jackson ImmunoResearch
Goat anti-mouse IgG1 (HRP)	IgG1 Fc y subtype-specific, secondary antibody, IgG isotype-specific ELISA	115-035-205	1:5,000	Jackson ImmunoResearch via Dianova
Goat anti-mouse IgG2a (HRP)	IgG2a Fc y subtype-specific secondary antibody, IgG isotype-specific ELISA	115-035-206	1:5,000	Jackson ImmunoResearch via Dianova
Goat anti-rabbit IgG HRP	ELISA	A0545-1ml	1:10,000	Sigma-Aldrich
GolgiPlug	ICS	555029	1:1,000	BD
GolgiStop	ICS	554724	1:1,500	BD
HBS-EP+ buffer 10×	SPR	BR100669	N/A	GE Healthcare
HEPES	1 M	15630-056	N/A	Gibco
Human SARS coronavirus spike S1 subunit antibody Anti-COVID-19-S1 Isotype: rabbit IgG	ELISA	40150-RP01	S1: 1:1,000 RBD: 1:2,000	Sino Biological
Insulin syringes	BD Micro-Fine™+, 30 G, 0.3 mL	324826	N/A	Becton Dickinson GmbH
Ionomycin	ICS	I9657	1 µg/mL	Sigma
Isoflurane	Anesthesia	9714675	N/A	Piramal Critical Care
Isotonic saline	Injection solution	06173569	N/A	Fresenius Kabi Deutschland GmbH
Lipofectamine® LTX & PLUS™	Transfection reagent	15338-100	N/A	Invitrogen
MACS LS columns	MACS	130-042-401	N/A	Miltenyi Biotec
MACS® MicroBeads	CD8a (Ly-2)/CD4 (L3T4)	130-117-044/130-117-043	N/A	Miltenyi Biotec
MaxiSorp plate	ELISA	439454	N/A	Thermo Scientific
MEM non-essential amino acids (NEAA) solution	100×	11140-035	1×	Gibco

Product name	Application/ specification	Article no.	Working dilution	Provider
Mouse IFN-γ ELISpot^{PLUS} kit	Kit for enumeration of cells secreting mouse IFN-γ	3321-4APT-2	N/A	Mabtech
Mouse IgG1-BIOT	Clone 15H6, isotype control for IgG-specific ELISA	0102-08	1:100	Southern Biotech via Biozol
Mouse IgG2a-BIOT	Clone HOPC-1, isotype control for IgG-specific ELISA	0103-08	1:100	Southern Biotech via Biozol
Mouse IgG-UNLB	ELISA	0107-01	Starting dilution 1:300	Southern Biotech
PBS powder	No calcium, no magnesium	L182-10	N/A	Merck KGaA
pcDNA3.1-derived expression plasmid	VSV vector production	V79020	N/A	Invitrogen
PE hamster anti-mouse CD3e clone 145-2C11	ICS	553064	1:200	BD
PE/Cy7 anti-mouse IFN-γ antibody, clone XMG1.2	ICS	505826	1:500	BioLegend
Penicillin-streptomycin	10,000 U/mL	15140-122	N/A	Gibco
Phosphate-buffered saline (PBS), powdered	ELISA	0780-10L	N/A	VWR International GmbH
Pipette tips	ep Dualfilter T.I.P.S.®, PCR clean und sterile, 0.1–10 µL/2–100 µL/50–1,000 µL/50–1,250 µL/0.1–5 mL	0030077512/0030077547/0030077555/0030077927/0030077750/0030078616	N/A	Eppendorf Vertrieb Deutschland GmbH
Phorbol 12-myristate 13-acetate (PMA)	ICS	P1585	0.5 µg/mL	Sigma
Potassium bicarbonate	$KHCO_3$	A2375,1000	N/A	AppliChem GmbH
ProcartaPlex assay	Bead-based, 11-plex T_H1/T_H2 mouse immunoassay	EPX110-20820-901	N/A	Thermo Fisher Scientific
Recombinant RBD protein SARS-CoV-2 (2019-nCoV) spike protein (RBD, Fc Tag)	ELISA	40592-V02H	100 ng/ 100 µL	SinoBiological
Reservoir	25 mL, 100 mL	613-1174/613-1171	N/A	VWR International GmbH
Roti Histofix, 4% formaldehyde	ICS	P087.4	2%	Carl Roth GmbH & Co. KG

Product name	Application/ specification	Article no.	Working dilution	Provider
RRPMI 1640 medium	GlutaMAX™ supplement	61870-010	N/A	Gibco
SARS-CoV-2 (2019-nCoV) spike antibody, rabbit Mab	ELISA	40150-R007	S1: 1:500 RBD: 1:1,000	Sino Biological
SARS-CoV-2 (2019-nCoV) spike RBD-Fc recombinant protein	ELISA	40592-V02H	100 ng/ 100 µL	Sino Biological
SARS-CoV-2 (2019-nCoV) spike RBD-His recombinant protein	SPR	40592-V08B	N/A	Sino Biological
SARS-CoV-2 (2019-nCoV) spike S1-His recombinant protein	ELISA, SPR	40591-V08H	N/A	Sino Biological
Serological pipettes	5 mL, 10 mL, 25 mL, 50 mL	606180/607 180/601180/ 768180	N/A	Greiner Bio-One GmbH
Single-use syringe	Injekt® Solo 5 mL	4606051V	N/A	B. Braun Melsungen AG
Sodium bicarbonate	ELISA	S5761	N/A	Sigma-Aldrich Chemie GmbH
Sodium carbonate	ELISA	S7795	N/A	Sigma-Aldrich Chemie GmbH
Sodium pyruvate	100 mM	11360-039	N/A	Gibco
Sterile filters	0.45 µm	514-4123	N/A	VWR International
Sulfuric acid 25% EMSURE®	ELISA	1007161000	N/A	VWR International GmbH
TMB One (3,3',5,5'-Tetramethylbenzidine) ready-to-use-solution	ELISA	4380A	N/A	Biotrend Chemikalien GmbH
Tween 20	ELISA	9127.1	N/A	Carl Roth GmbH & Co. KG
Vero-76 cells	Pseudovirus titration	CRL-1587	N/A	ATCC
Vi-CELL™ XR Quad Pak	For Vi-CELL™ XR Cell Viability Analyzer	383722	N/A	Beckman Coulter GmbH
VSV-ΔG-GFP vector	VSV vector production	EH1004	N/A	Kerafast

Table 3: Equipment

Product name	Application	Provider
Biacore T200	SPR analysis	Cytiva
Vi-CELL™ XR Cell Viability Analyzer	Splenocyte count	Beckman Coulter GmbH
CTL ImmunoSpot S6 Core Analyzer	ELISpot plate reader	Cellular Technology Ltd.
BioTek Epoch reader	ELISA plate reader	BioTek
IncuCyte Live Cell Analysis system	pVNT	Sartorius
Celesta	Flow cytometry analysis (ICS)	BD

Table 4: Software

Product name	Application	Provider
Biacore T200 Evaluation Software 3.1	SPR analysis	Cytiva
Excel	Animal monitoring, raw data	Microsoft Corp.
GraphPad Prism 8	Analysis of ELISpot, ELISA, and pVNT	GraphPad Software Inc.
Gen5 software 3.0.9	ELISA plate read out	BioTek
ImmunoCapture 7.0.7.0	ELISpot analysis	Cellular Technology Ltd.
ImmunoSpot® analysis software version 57.0.17.0	ELISpot analysis	Cellular Technology Ltd.
IncuCyte Live Cell Analysis system	pVNT	Sartorius
BD FACSDiva software version 8.0.1.1	Flow cytometry analysis (ICS)	BD

Table 5: Peptide pools for stimulation of splenocytes for ELISpot assays

S protein-specific peptides	
Name	**Sequence**
2019-nCoV S.wt With a total of 315 overlapping peptides (Format 15/11) GenBank: QHD43416.1 Batch: 43000LHB-1 and 43000LHB-2	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEF RVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPI NLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAA AYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTS NFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLY NSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCG PKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRD PQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRA RSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCT MYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTP PIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTY VTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHG VVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYE PQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVD LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLG FIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHY T
RBD-specific peptides	
Name	**Sequence**
2019-nCoV RBD With a total of 48 overlapping peptides (Format 15/11)	VRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKC YGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGC VIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVE GFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPK
Irrelevant peptide control	
Name	**Sequence**
AH-1	SPSYVYHQF

4.5 Methods

4.5.1 Animal Care

4.5.1.1 General Information

BALB/c mice were delivered at the age of at least six weeks. Delivered mice were used for experiments after approximately one week of acclimatization. All experiments and protocols were approved by the local authorities (local animal welfare committee), conducted according to the Federation of European Laboratory Animal Science Associations (FELASA) recommendations and in compliance with the German animal welfare act and Directive 2010/63/EU. Only animals with an unobjectionable health status were selected for testing procedures.

All animals were registered upon arrival in the lab animal colony management system PyRAT (Scionics Computer Innovation GmbH, Dresden, Germany) and tracked until death. Each cage was labeled with a cage card indicating the mouse strain, sex, date of birth, and number of animals per cage. At the start of an experiment additional information was added such as the project and license number, the start of the experiment and details on interventions. Where necessary for identification, animals were arbitrarily numbered with earmarks.

4.5.1.2 Housing Conditions and Husbandry

Mice were housed at BioNTech SE's animal facility (An der Goldgrube 12, 55131 Mainz) under barrier and specific-pathogen-free (SPF) conditions in individually ventilated cages (Sealsafe GM500 IVC Green Line, TECNIPLAST, Hohenpeißenberg, Germany; 500 cm²) with a maximum of five animals per cage. The temperature and relative humidity in the cages and animal unit were kept at 20-24°C and 45-55%, respectively, and the air change (AC) rate in the cages was 75 AC/h. Cages contained dust-free bedding made of debarked chopped aspen wood (Abedd LAB & VET Service GmbH, Vienna, Austria, product code: LTE E-001) and additional nesting material was changed weekly. Autoclaved ssniff M-Z food (sniff Spezialdiäten GmbH, Soest, Germany; product code: V1124) and autoclaved tap water were provided *ad libitum* and changed at least once weekly. All materials were autoclaved prior to use.

4.5.2 Animal Monitoring

Routine animal monitoring was carried out daily and included inspection for dead mice and control of food and water supplies. The health of each mouse was closely assessed at least once weekly and the results documented in health monitoring sheets (see Appendix 1: Animal Monitoring - Observations). The general physical condition of the mice was assessed according to the following parameters:

- Body weight change
- Macroscopic assessment of activity level/behavior
- Macroscopic assessment of general discomfort: drop in body temperature determined by touch and by visual inspection of ears and paws (ears and paws appear pink in a healthy mouse, white in a mouse with discomfort indicates reduced blood circulation)
- Macroscopic assessment of fur condition and appearance of eyes, inspection of body cavities/fluids
- Macroscopic assessment of irregularities in breathing ability
- Indication of pain
- Macroscopic assessment for signs of automutilation and/or fighting

Details on animal monitoring criteria are shown in Appendix 1: Animal Monitoring - Observations, Table 9.

4.5.3 Animal Treatment

4.5.3.1 Treatment Schedule, Route of Administration, and Dose

The test compound was administered i.m. once at three different doses (0.2 µg, 1 µg, or 5 µg per animal) to the three test groups of mice on day 0. The control group was treated with buffer alone.

4.5.3.2 Immunization

Following anesthesia by inhalation of 2.5% isoflurane in oxygen, the injection site on the hind leg of the mouse was shaved for immunization. Buffer or dissolved test item was applied i.m. into the *musculus gastrocnemius* in a volume of 20 µL. After immunization and a short recovery phase from anesthesia, the mice were observed for any immediate signs of discomfort due to the immunization procedure.

4.5.3.3 Blood Sampling via the Retro-Orbital Venous Plexus or *Vena Facialis*

Blood was sampled via the retro-orbital venous plexus according to SOP-030-074. In short, mice were anesthetized by inhalation of 2.5% isoflurane in oxygen and tightly held for blood collection. A thin glass capillary (29 G) was inserted gently through the retro-orbital sinus membrane and blood was collected into an appropriate plastic tube (Sarstedt, Z-gel included for clotting activation). After careful removal of the glass capillary, the restraining hold on the mouse was loosened. Alternatively, blood collection took place via the *vena facialis* according to SOP-030-074. In short, without prior anesthesia, mice were tightly held for blood collection, and the *vena facialis* was punctured using a lancet in a precise and short movement. Blood was collected into an appropriate plastic tube (Sarstedt, Z-gel included for clotting activation), and then

the restraining hold on the mouse was loosened. Blood samples were centrifuged at 10,000 ×g and RT for 5 min and serum transferred to a pre-labeled 0.5 mL reagent tube for use in subsequent downstream assays or storage at -20°C.

4.5.4 Endpoint of Experiment/Termination Criteria

Animals were euthanized in accordance with §4 of the German animal welfare act and the recommendation of the German Society of Laboratory Animal Science (GV-SOLAS) by cervical di location or by e po ure to carbon dio ide Additionally, termination criteria were applied according to the specification within the respective animal test approval as listed below. Body weight losses exceeding 20%, or a high severity level in any of the parameters found in Section 4.5.2 were on their own sufficient reason for immediate euthanasia.

4.5.4.1 Dissection of Animals and Organ Collection

Following euthanasia, mice were disinfected with 70% ethanol and the dissection was performed starting with an abdominal incision. The spleen was collected and stored in DPBS on ice for subsequent splenocyte preparation.

4.5.5 ELISA

Serum samples were tested in 96-well plates for their S-specific antibody concentration based on SOP-030-112 (with minor modifications as described below). Briefly, for the time points 7, 14, and 21 days after immunization, a screening analysis was performed and for day 14 and 28, serum samples were analyzed by endpoint titration.

1. Coat each well of a MaxiSorp plate with 100 ng/100 µL recombinant protein per well or isotype controls according to plate layout.
   - Coating buffer: 50 mM sodium carbonate buffer (1.696 g Na_2CO_3 + 2.856 g $NaHCO_3$, top up to 1 L distilled H_2O, pH 9.6 (pH adjustment not needed))
2. Cover plates and incubate at 4°C o/n.
3. Wash three times with 300 µL/well PBS with Tween (PBS-T).
4. Block all wells with 1xBB, 250 µL/well.
5. Incubate at 37°C for 1 h on shaker.
6. Wash three times with 300 µL/well PBS-T.
7. Dilute primary antibodies (samples and positive control) according to schedule.
8. Incubate at 37°C for 1 h on shaker.
9. Wash three times with 300 µL/well PBS-T.
10. Dilute the secondary antibodies according to calculations.
11. Incubate at 37°C for 45 min on shaker.
12. Wash three times with 300 µL/well PBS-T.
13. Add 100 µL/well TMB substrate.
14. Incubate 8 min at RT (clear->blue).

15. Stop the reaction with 100 µL 25% sulfuric acid. (blue -> yellow).
16. Read on plate reader (450 nm, reference: 620 nm).

For concentration analysis, the signal of the specific samples was correlated to the isotype control. For analysis of IgG subtypes, the mean ΔOD 450-620 nm per group was calculated and the ratio of IgG2a:IgG1 ratio was calculated.

For reciprocal serum endpoint titer, the serum dilution that emitted the OD exceeding 4-fold background was used. The background was defined as the OD signal given by the recombinant protein incubated with the secondary detection anti-mouse IgG antibody only.

4.5.6 Surface Plasmon Resonance Spectroscopy

Binding kinetics of murine S1- and RBD-specific serum IgGs was determined using a Biacore T200 device with HBS-EP running buffer at 25°C. Carboxyl groups on the CM5 sensor chip matrix were activated with a mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimidehydrochloride (EDC) and N-hydroxysuccinimide (NHS) to form active esters for the reaction with amine groups. Anti-mouse-Fc-antibody was diluted in 10 mM sodium acetate buffer pH 5 (30 µg/mL) for covalent coupling to immobilization level of ~10,000 response units (RU). Free NHS esters on the sensor surface were deactivated with ethanolamine.

Mouse serum was diluted 1:50 in HBS-EP buffer and applied at 10 µL/min for 30 seconds to the active flow cell for capture by immobilized antibody, while the reference flow cell was treated with buffer. Binding analysis of captured murine IgG antibodies to S1-His or RBD-His was performed using a multi-cycle kinetic method with concentrations ranging from 25 to 400 nM or 1.5625 to 50 nM, respectively. An association period of 180 seconds was followed by a dissociation period of 600 seconds with a constant flow rate of 40 µL/min and a final regeneration step. Binding kinetics were calculated using a 1:1 Langmuir global kinetic fit model.

4.5.7 Pseudovirus-based Neutralization Test

For analyzing the amount of functional nAbs in the serum samples, pVNTs were performed.

4.5.7.1 Production of SARS-CoV-2-S Pseudotyped VSV Vector

Replication-deficient vesicular stomatitis virus (VSV) that lacks the genetic information for the VSV envelope glycoprotein VSV-G but contains an open reading frame (ORF) for green fluorescent protein (GFP) was used for SARS-CoV-2-S pseudovirus generation. VSV pseudotypes were generated according to a published protocol (Hoffmann et al. 2020).

In brief, HEK293T/17 cells cultured in DMEM supplemented with 10% FBS were transfected with a pcDNA3.1-derived expression plasmid (Invitrogen) coding for the SARS-CoV-2 spike protein (GenBank ID: QHD43416.1) with shortened cytoplasmic tail, i.e., pSARS-CoV-2-S-CΔ19, using Lipofectamine® LTX & PLUS™ Reagent (Invitrogen) following the manufacturer's instructions. The cytoplasmic tail was truncated for the 19 C-terminal amino acids to facilitate a more efficient integration of SARS-CoV-2-S into VSV virions analogous to SARS-CoV-2-S pseudotyped VSV (Fukushi et al. 2005). At 24 h post transfection, cells were inoculated with VSV-G transcomplemented VSV-ΔG-GFP vector (Indiana strain, *de novo* generated by reverse genetics from plasmid (Lawson et al. 1995)) at a multiplicity of infection (MOI) of three and incubated for 2 h at 37°C and 5% CO_2. Next, the inoculum was removed, cells were washed with PBS, and standard culture medium which contained 0.5 µg/mL anti-VSV-G antibody (clone 8G5F11) was added to neutralize residual input virus. Twenty-four hours after infection, VSV/SARS-CoV-2-S pseudovirus-containing supernatants were harvested, filtered (0.45 µm) and stored at -80°C in aliquots until further use.

4.5.7.2 Titration of VSV/SARS-CoV-2-S Pseudovirus

For titration of VSV/SARS-CoV-2-S pseudovirus, Vero-76 cells (ATCC) were thawed according to SOP-030-041, diluted to 2.67×10^5 cells/mL in assay medium (DMEM and 10% FBS) and seeded in 96-well flat-bottom plates at 4×10^4 cells per well. Cells were incubated for 4 to 6 h at 37°C and 7.5% CO_2. Meanwhile, two-fold, eight-step serial dilutions were prepared in 96-well V-bottom plates beginning with undiluted pseudovirus supernatant. Vero-76 wells were inoculated with 50 µL of the diluted pseudovirus supernatant and incubated for 16 to 24 h at 37°C and 7.5% CO_2. Each dilution was tested in duplicate wells. After the incubation, the cell culture plates were removed from the incubator, placed in an IncuCyte Live Cell Analysis system (Essen Bioscience) and equilibrated for 30 min prior to the analysis. Whole well scanning for brightfield and GFP fluorescence was performed using a 4× objective. The number of infected GFP-fluorescent cells per well was plotted as a function of pseudovirus supernatant dilution using GraphPad Prism. Data (x = logx) were fitted with linear regression and the derived slope and y-intercept used to calculate the amount of viral supernatant needed to obtain 144 infected cells/96-well (20% excess for virus neutralization test included).

4.5.7.3 Pseudovirus-based Neutralization Test

Sera from blood samples collected 14, 21 and 28 days after immunization were tested using the VSV/SARS-CoV-2-S pseudovirus neutralization test (pVNT). For the pVNT assay, Vero-76 cells were thawed according to SOP-030-041, diluted to 2.67×10^5 cells/mL in assay medium (DMEM and 10% FBS) and seeded in 96-well flat-bottom plates at 4×10^4 cells per well. Cells were incubated for 4 to 6 h at 37°C and 7.5% CO_2. Initial dilutions of mouse serum samples were prepared by adding 10 µL of serum

to 50 µL assay medium in a 96-well V-bottom plate. Seven additional dilutions were subsequently prepared in two-fold dilution steps, by iteratively transferring 30 µL of diluted sera to wells containing 30 µL assay medium. VSV/SARS-CoV-2 pseudovirus was thawed and diluted to obtain 120 infected cells/25 µL (4.8×10^3 infectious units [IU]/mL). 30 µL of diluted pseudovirus (corresponds to 144 infected cells; see Section 4.5.7.2) was added to the wells containing the serum dilution series. Pseudovirus/serum dilution mix was incubated for 5 min at RT on a microplate shaker at 750 rpm, and additional 5 min at RT without agitation. Pseudovirus/serum dilution mix was then added to the seeded Vero-76 cells (50 µL mix per well, MOI:0.003), followed by incubation for 16 to 24 h at 37°C and 5% CO_2. Each dilution of serum samples was tested in duplicate wells. Vero-76 cells incubated with pseudovirus in the absence of mouse sera were used as positive controls. Vero-76 cells incubated without pseudovirus were used as negative controls. After the incubation, the cell culture plates were removed from the incubator, placed in an IncuCyte Live Cell Analysis system and incubated for 30 min prior to the analysis. Whole well scanning for brightfield and GFP fluorescence was performed using a 4× objective. To calculate the neutralizing titer, infected GFP-positive cell number per well was compared with the no-serum pseudovirus positive control. Mean values of the no-serum pseudovirus positive control multiplied by 0.5 represent the pseudovirus neutralization 50% (pVN_{50}); mean values of the no-serum pseudovirus positive control multiplied by 0.1 represent the pseudovirus neutralization 90%. Serum samples with mean values below this cut-off exhibit >50% or >90% virus neutralization activity, respectively.

4.5.8 Preparation of Splenocytes

The single cell suspensions from collected spleens were prepared according to SOP-030-078. To this end, the spleens were squeezed through 70 µm cell meshes using the plunger of a syringe to release the splenocytes into a tube. Splenocytes were washed with an excess volume of DPBS followed by centrifugation at 300 × g for 6 min at RT and discarding the supernatants. Erythrocytes were then lysed with erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 5 min at RT. The reaction was stopped with an excess volume of DPBS. After another washing step, cells were resuspended in medium (10% FBS, 1% NEAA, 1% sodium pyruvate, 0.5% penicillin/streptomycin), passed through a 70 µm cell mesh again, counted according to SOP-010-028, and stored short-term at 37°C for use on the same day or frozen in liquid nitrogen, resuspended in 1 mL FBS/10% DMSO. For the use of frozen splenocytes in ELISpot analysis after thawing, the amount of cells per well was doubled (1×10^6 cells). Immediately after thawing, pre-warmed (RT) PBS was added to splenocytes. Two washing steps using pre-warmed PBS to remove DMSO from freezing process were performed and splenocytes were counted according to SOP-010-028. Splenocytes were stored short-term at 37°C for further use.

4.5.9 ELISpot Assay

ELISpot assays with fresh or frozen splenocytes were performed according to SOP-030-110 (with minor modifications as described below) using the mouse IFN-γ ELISpot^{PLUS} kit. Briefly, 96-well ELISpot plates were washed with PBS and blocked with medium for at least 30 min at 37°C. 100 µL of the splenocyte solution (fresh cells: 5×10^5 cells; frozen cells: 1×10^6 cells) were transferred to the respective well of the 96-well ELISpot plate. Another 100 µL of overlapping peptide pools or controls were added in the following concentrations:

- overlapping peptide mix PepMix™ against SARS-CoV-2 S.wt: 0.1 µg/mL final concentration per peptide
- overlapping peptide mix PepMix™ against SARS-CoV-2 RBD: 0.1 µg/mL final concentration per peptide
- irrelevant peptide (AH-1): 4 µg/mL
- Concanavalin A (ConA): 2 µg/mL

For positive control, the splenocytes were stimulated with ConA, for a non-stimulation control only medium was added and as a negative control to detect unspecific background signals, the irrelevant peptide was added (AH-1). Plates were incubated overnight in a 37°C humidified incubator with 5% CO_2 and after approximately 18 h, cells were removed from the plates and the detection protocol of spots was initiated. To this end, the detection antibody, Streptavidin-ALP, and the ready-to-use substrate were added to the wells according to the manufacturer's protocol. After plate drying for 2–3 h under the laminar flow, an ELISpot plate reader (ImmunoSpot® S6 Core Analyzer, CTL) was used to count and analyze spot numbers per well.

4.5.9.1 Subtyping of $CD8^+$ versus $CD4^+$ T-cell Responses

This method was performed with fresh splenocytes (non-frozen). $CD8^+$ or $CD4^+$ T cells were isolated from splenocyte cell suspensions using MACS® MicroBeads (CD8a (Ly-2) or CD4 (L3T4)) according to the manufacturer's instructions. Labeled cells were eluted from MACS LS columns, centrifuged (5 min at 460 ×g) and taken up at a concentration of 1×10^6 cells/mL in medium. 100 µL of $CD8^+$ or $CD4^+$ T cells were subsequently re-stimulated by addition of 50 µL peptide solution (control peptide AH-1 (2 µg/mL), RBD peptide mix (0.1 µg/mL per peptide) or S peptide mix (0.1 µg/mL per peptide)) and 50 µL of bone marrow-derived dendritic cells (1×10^6 cells/mL, cells were frozen at -80°C prior use and prepared from BALB/c mice according to SOP-030-080) in an IFN-γ ELISpot assay (SOP-030-110). Each condition was tested in duplicate.

4.5.10 Luminex Assay

1×10^6 previously frozen splenocytes in 100 µL DC medium (part of SOP-030-110) were transferred to a 96-well flat-bottom cell culture plates. 100 µL of an overlapping peptide pool or controls were added in the following concentrations:

- overlapping peptide mix PepMix™ against SARS-CoV-2 S.wt: 0.1 or 0.03 µg/mL final concentration per peptide (equal to 31.5 or 9.6 µg/mL total peptide)
- overlapping peptide mix PepMix™ against SARS-CoV-2 RBD: 0.66 or 0.2 µg/mL final concentration per peptide (equal to 31.5 or 9.6 µg/mL total peptide)
- PMA: 1 µg/mL and ionomycin: 2 µg/mL

The plates were incubated for 48 h and supernatant thereafter was harvested for cytokine profiling. Cytokine concentrations in supernatants of the re-stimulated splenocytes were determined using a bead-based, 11-plex T_H1/T_H2 mouse ProcartaPlex immunoassay according to the manufacturer's instructions. Fluorescence was measured with the Bioplex200 System (Bio-Rad) and analyzed with ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific). The following analytes were measured: IFN-γ, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, TNF-α, GM-CSF, and IL-18.

4.5.11 Intracellular Cytokine Staining

Briefly, 5×10^5 fresh splenocytes in 100 µL DC medium (part of SOP-030-110) were transferred to 96-well flat-bottom cell culture plates. Finally, 100 µL of an overlapping peptide pool or controls were added in the following concentrations:

- overlapping peptide mix PepMix™ against SARS-CoV-2 S.wt: 0.1 µg/mL final concentration per peptide (equal to 31.5 µg/mL total peptide)
- overlapping peptide mix PepMix™ against SARS-CoV-2 RBD: 0.1 µg/mL final concentration per peptide (equal to 4.8 µg/mL total peptide)
- PMA: 1 µg/mL and ionomycin: 2 µg/mL

As a non-stimulation control, only medium was added to detect unspecific background signals. Plates were incubated for 1 h in a 37°C humidified incubator with 5% CO_2 before adding a GolgiStop+GolgiPlug. After another 4 h, cells were harvested and transferred to a 96-well, V-bottom plate for flow cytometry staining. After the staining procedure, cells were solved in 100 µL FACS buffer (PBS + 0.1% BSA) for flow cytometry analysis using a FACS Celesta (BD).

A detailed protocol is presented in Appendix 5: Detailed ICS Protocol.

4.5.12 Statistical Analysis

GraphPad Prism 8 Software (La Jolla, USA) was used for statistical analysis and figure generation. All test groups were compared to the buffer control group by a one-way analysis of variance (ANOVA) on each measurement day as described in the respective results section. For Luminex assays, statistical significance was assessed by mixed-effects analysis/Sidak's comparison.

5 RESULTS

5.1 ELISA

5.1.1 Whole IgG ELISA

IgGs against recombinant S1 protein or RBD were detected by ELISA analysis in serum samples obtained on study days 7, 14, and 21. Statistical significance was assessed by one-way ANOVA and Dunnett's multiple comparisons test.

Before immunization, no S1 protein- or RBD-specific IgGs were detected (Pre-treatment, Figure 2, Figure 3). Treatment with BNT162b2 induced the formation of IgGs specific for S1 protein and RBD, while these antibodies were not detected in samples from buffer control animals independent of the day of sample collection. A dose-dependent increase in S1-specific IgGs was observed on all study days (Figure 2), with statistically significant differences between the treatment groups and the buffer control group ($p < 0.0001$ for all doses and test days).

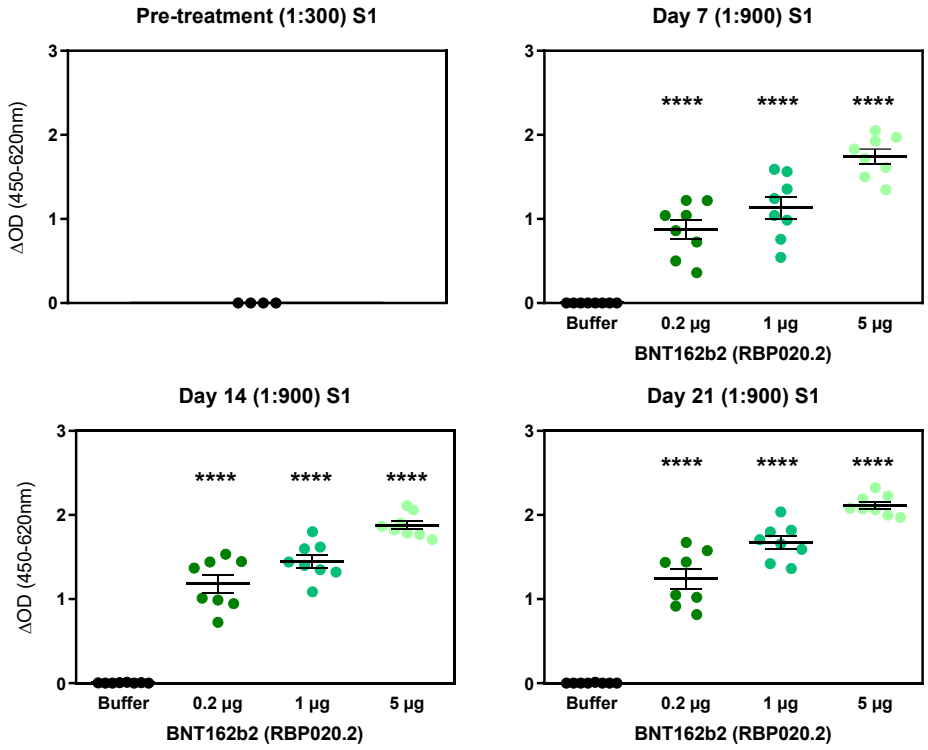

Figure 2: ELISA screening analysis on days 7, 14, and 21 against the recombinant S1 protein

ELISA was performed using serum samples collected on days 7, 14, and 21 after immunization to analyze antibody responses. The serum samples were tested against the S1 protein. Individual ΔOD values for each mouse (measured in duplicates) are shown by dots; group mean values are indicated by horizontal bars (±SEM). **** $p < 0.0001$.

All test groups showed a statistically significant increase in RBD-specific IgGs compared to buffer control (Figure 3; $p < 0.0001$ for all doses and test days).

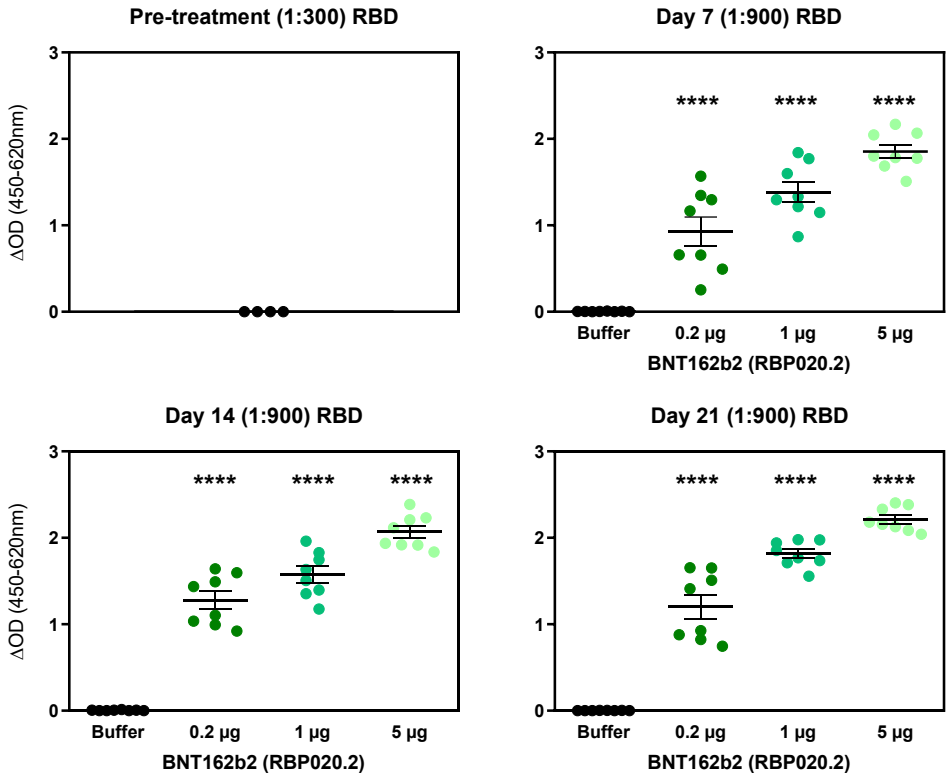

Figure 3: ELISA screening analysis on days 7, 14, and 21 against the recombinant RBD

ELISA was performed using serum samples collected on days 7, 14, and 21 after immunization to analyze antibody responses. The serum samples were tested against the receptor-binding domain (RBD). Individual ΔOD values for each mouse (measured in duplicates) are shown by dots; group mean values are indicated by horizontal bars (±SEM). **** $p < 0.0001$.

ELISA endpoint titration was performed on day 28 after immunization to analyze antibody responses (Figure 4A, B).

Antibody concentrations in the serum samples were calculated for the individual sampling days and the kinetics of IgGs against S1 and RBD proteins is shown in Figure 5. Antibody concentrations against S1 (Figure 5A) and RBD (Figure 5B) increased in a dose-dependent manner over time in the test groups. Statistical significance of the differences in IgG concentrations between the test groups and the control group was assessed by one-way ANOVA with Dunnett's multiple comparison post-test on day 28.

The differences in concentrations of IgGs against S1 and RBD in the test groups compared to the buffer control group were statistically significant (S1: $p = 0.0259$ for 0.2 µg, $p < 0.0001$ for 1 µg and 5 µg; RBD: $p = 0.0072$ for 0.2 µg, $p < 0.0001$ for 1 µg and 5 µg) on day 28.

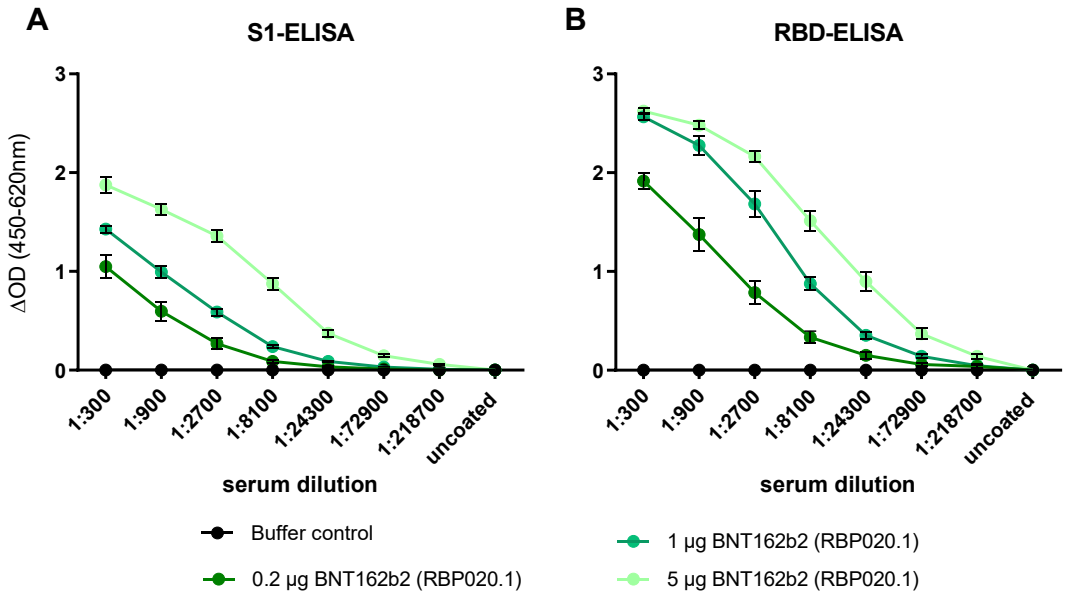

Figure 4: ELISA endpoint titration on day 28

Endpoint titration was performed on day 28 after immunization to analyze antibody responses. The serum samples were tested against the S1 protein (A) and RBD (B). Group mean values (±SEM) are shown.

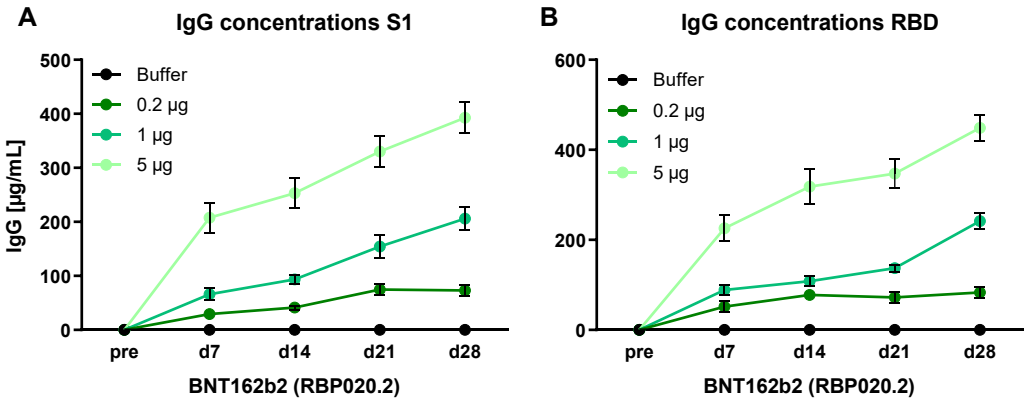

Figure 5: Kinetics of the antibody concentration against the viral antigen

For individual ΔOD values, the antibody concentrations in the serum samples were calculated. The serum samples were tested against (A) the S1 protein and (B) RBD. Group mean antibody concentrations are shown (±SEM).

Furthermore, to calculate the reciprocal serum endpoint titer of antibodies we performed an endpoint titration for day 14 and 28 samples after immunization exceeding the previously shown dilution steps (Figure 6A, B).

The reciprocal serum endpoint titer was defined as the first highest dilution step which emitted an OD exceeding the background signal four-times as shown. Reciprocal serum endpoint titer against S1 (Figure 7A) and RBD (Figure 7B) were high already 14 days after immunization and increased in a dose-dependent manner over time in the test groups. Statistical significance of the differences in IgG concentrations between the test groups and the control group was assessed by a one-way ANOVA with Tukey's multiple comparison post-test.

The differences in titers of IgGs against S1 and RBD in the test groups compared to the buffer control group were statistically significant (S1, day 28: $p = 0.0082$ for 1 µg, $p < 0.0001$ for 5 µg; RBD, day 14: $p < 0.0001$ for 5 µg and day 28: $p = 0.0109$ for 1 µg, $p < 0.0001$ for 5 µg).

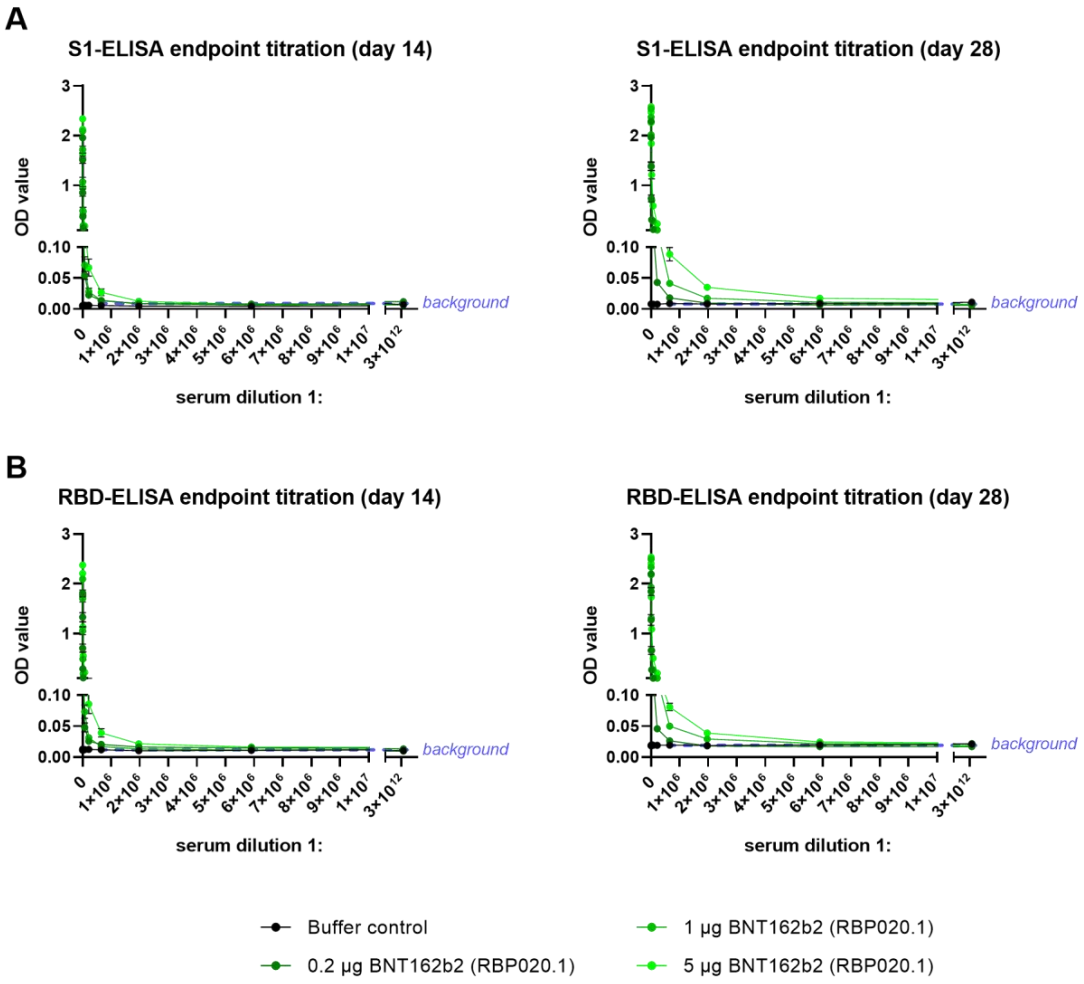

Figure 6: ELISA endpoint titration (long titration)

Endpoint titration against the S1 protein (A) and RBD (B) was performed on day 14 (left) and 28 (right) after immunization to analyze reciprocal serum endpoint titer of antibodies. Group mean values (±SEM) are shown; samples were measured in duplicates. Background was defined as the OD value of the recombinant protein incubated with the secondary anti-mouse IgG detection antibody only and included in the graphs (blue dotted line; n = 8).

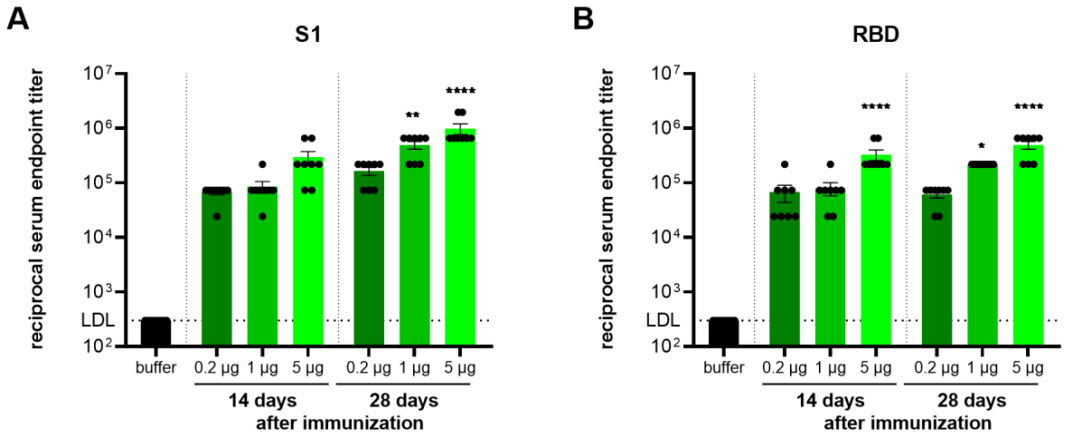

Figure 7: Reciprocal serum endpoint titer at day 14 and 28 after immunization

For individual OD values, the reciprocal serum endpoint titer was calculated. The serum samples were tested against (A) the S1 protein and (B) RBD. Group mean titer are shown (±SEM). Significance compared to buffer control is included, * $p \leq 0.05$, ** $p \leq 0.01$, **** $p < 0.0001$; LDL = lower detection limit.

5.1.2 IgG Subtype-specific ELISA

IgG1 and IgG2a subtypes of anti-S1 antibodies were analyzed by IgG subtype-specific ELISA in serum samples obtained on study day 28. Statistical significance was assessed by one-way ANOVA followed by a Dunnett's multiple comparison post-test.

Treatment with BNT162b2 induced the formation of IgG1 and IgG2a specific for S1 protein, while these antibodies were not detected in samples from buffer control animals independent of the day of sample collection (Figure 8).

On day 28, all dose groups displayed significantly higher group mean ΔOD values for IgG1 and IgG2 antibodies than the control animals (IgG1: $p < 0.0001$ for all doses, IgG2a: $p = 0.0020$ for 0.2 µg, $p < 0.0001$ for 1 µg and 5 µg).

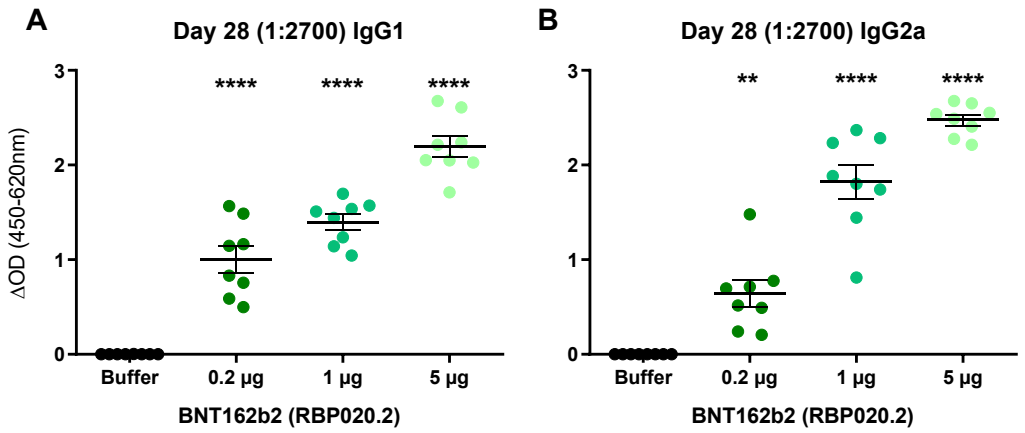

Figure 8: IgG subtype-specific ELISA on day 28

ELISA was performed using serum samples collected on day 28 after immunization to analyze (A) IgG1 and (B) IgG2a responses. The serum samples were tested against theS1 protein. Individual ΔOD values for each mouse (measured in duplicates) are shown by dots; group mean values are indicated by horizontal bars (±SEM). ** $p \leq 0.01$, **** $p < 0.0001$.

ELISA endpoint titration was performed on day 28 after immunization to analyze IgG1 and IgG2a responses (Figure 9A, B).

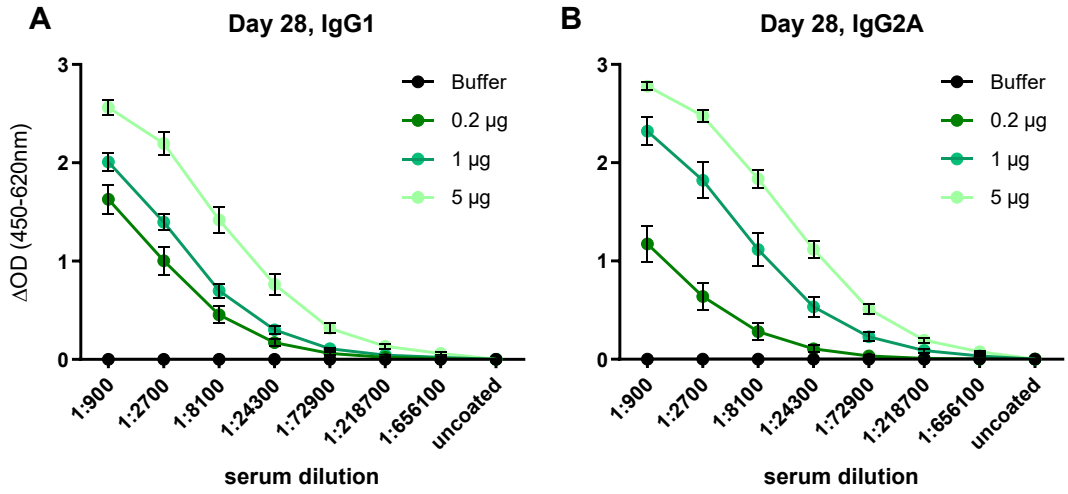

Figure 9: ELISA endpoint titration on day 28 (IgG subtypes)

Endpoint titration was performed on day 28 after immunization to analyze IgG1 (A) and IgG2a (B) responses. Group mean values (±SEM) are shown.

5.1.3 IgG2a/IgG1 Ratio

To analyze the ratio between the two IgG subtypes, the ΔOD values were used. Antibody ratios in the serum samples were calculated for day 28 (Figure 10). Statistical significance was assessed by one-way ANOVA followed by Tukey's multiple comparison post-test to compare all test groups with each other.

While the two higher doses induced a balanced IgG2a/IgG1 response, the lowest dose induced a higher ignal for IgG1 than IgG2a The difference between the group treated with 0.2 µg and the groups treated with 1 µg and 5 µg were statistically significant (p = 0.0004 for 0.2 µg vs 1 µg, p = 0.0041 for 0.2 µg vs 5 µg).

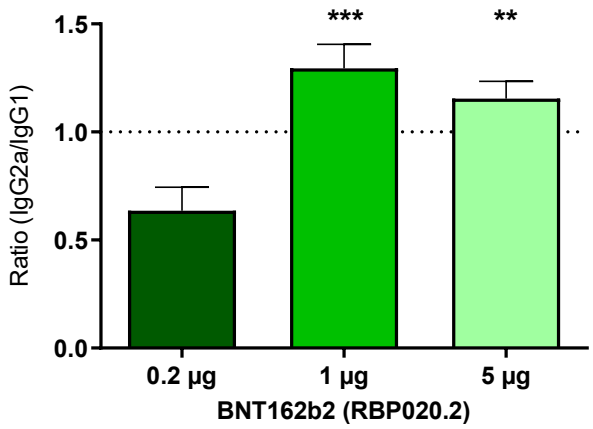

Figure 10: IgG2a/IgG1 subtype ratio on day 28

Based on the 1:2,700 dilution step (see Figure 9), the ΔOD the ΔOD for every single sample were used to calculate the ratio of IgG2a and IgG1. For this purpose, the ΔOD value of IgG2a was divided by the ΔOD values of IgG1 per mouse. Group mean values (±SEM) are shown. The value of "1" in the graph would give the equal signal between the two subtypes while ratio > 1 mirror a higher IgG2a subtype detection. ** p ≤ 0.01, *** p ≤ 0.001.

5.2 Binding Kinetics of Antigen-specific IgGs Using SPR

To obtain kinetic and affinity information about the binding of vaccine-elicited IgG to SARS-CoV-2 S1 fragment and RBD, SPR spectroscopy was conducted. Whole IgG from sera (n = 8) generated at 28 days after immunization with 5 µg BNT162b2 was captured by high-affinity anti-IgG antibody immobilized on the sensor chip surface. Binding analysis of captured murine IgG antibodies to recombinant S1-His or RBD-His protein was performed using a multi-cycle kinetic method with concentrations ranging from 25 to 400 nM S1-His or 1.5625 to 50 nM RBD-His. Kinetic parameters were calculated by fitting the sensorgram curves with a 1:1 Langmuir global kinetic fit model.

At day 28 after immunization, vaccine-elicited IgG had a strong binding affinity for S1-His (geometric mean K_D = 12 nM), with affinities ranging from 8.06 nM to 34.5 nM across the 8 serum samples tested (Figure 11A, Table 6). Somewhat higher binding affinity was detected for RBD-His (geometric mean K_D = 0.99 nM), with affinities ranging from 0.48 nM to 2.78 nM (Figure 11A, Table 7). Binding to S1-His and RBD-His can be characterized by a comparable low dissociation rate constant (geometric mean $k_{off} = 4\times10^{-4}$ s^{-1} vs. 5.97×10^{-4} s^{-1}). However, association of RBD-His to captured IgG was approximately 20-fold faster (geometric mean $k_{on} = 6.02\times10^{5}$ $M^{-1}s^{-1}$ vs. 3.33×10^{4} $M^{-1}s^{-1}$).

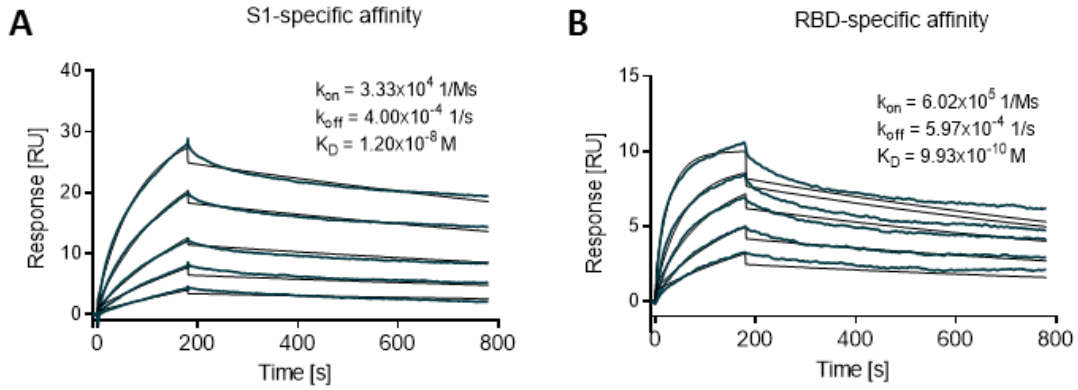

Figure 11: Binding affinities of vaccine-elicited IgG for S1-His and RBD-His protein

Representative SPR sensorgram of the binding kinetics of recombinant S1-His (A) and RBD-His protein (B) to immobilized mouse IgG from serum 28 days after immunization with 5 µg BNT162b2 (n=8). Actual binding (dark blue) and the best fit of the data to a 1:1 binding model (thin line in black) is shown.

Table 6: Summary of binding kinetic parameters of vaccine-elicited IgG for S1-His

Animal no.	k_{on} [1/Ms]	k_{off} [1/s]	K_D [nM]
4-1	2.62E+04	9.05E-04	34.5
4-2	3.36E+04	5.70E-04	17.0
4-3	2.91E+04	4.92E-04	16.9
4-4	4.78E+04	5.95E-04	12.5
4-5	2.94E+04	4.54E-04	15.4
4-6	2.45E+04	2.95E-04	12.0
4-7	2.91E+04	3.16E-04	10.9
4-8	3.36E+04	2.71E-04	8.06
Geometric mean	3.33E+04	4.00E-04	12.0

Table 7: Summary of binding kinetic parameters of vaccine-elicited IgG for RBD-His

Animal no.	k_{on} [1/Ms]	k_{off} [1/s]	K_D [nM]
4-1	4.35E+05	6.79E-04	1.56
4-2	2.89E+05	8.04E-04	2.78
4-3	6.64E+05	7.23E-04	1.09
4-4	4.82E+05	5.82E-04	1.21
4-5	4.64E+05	6.26E-04	1.35
4-6	8.46E+05	4.07E-04	0.481
4-7	6.36E+05	5.55E-04	0.873
4-8	1.06E+06	5.42E-04	0.512
Geometric mean	6.02E+05	5.97E-04	0.993

5.3 Pseudovirus-based Neutralization Test

Virus-neutralizing antibodies in serum samples obtained on study days 14, 21, and 28 were detected by pVNT. Statistical significance was assessed by one-way ANOVA with Dunnett's multiple comparison post-test.

Treatment with all tested uRNA doses induced the formation of virus-neutralizing antibodies with temporally increasing pVN_{50} titers (Figure 12). On day 14, several samples from animals treated with 0.2 µg modRNA displayed pVN_{50} titers that were below the lower limit of quantification. Significantly higher pVN_{50} titers were measured in samples from animals treated with the high dose of 5 µg RNA than in buffer control samples ($p = 0.0010$). On days 21 and 28, the differences of the groups treated with 1 µg and 5 µg BNT162b2 compared to the buffer control group were statistically significant (day 21: $p = 0.0036$ for 1 µg, $p < 0.0001$ for 5 µg; day 28: $p < 0.0001$ for 1 µg and 5 µg).

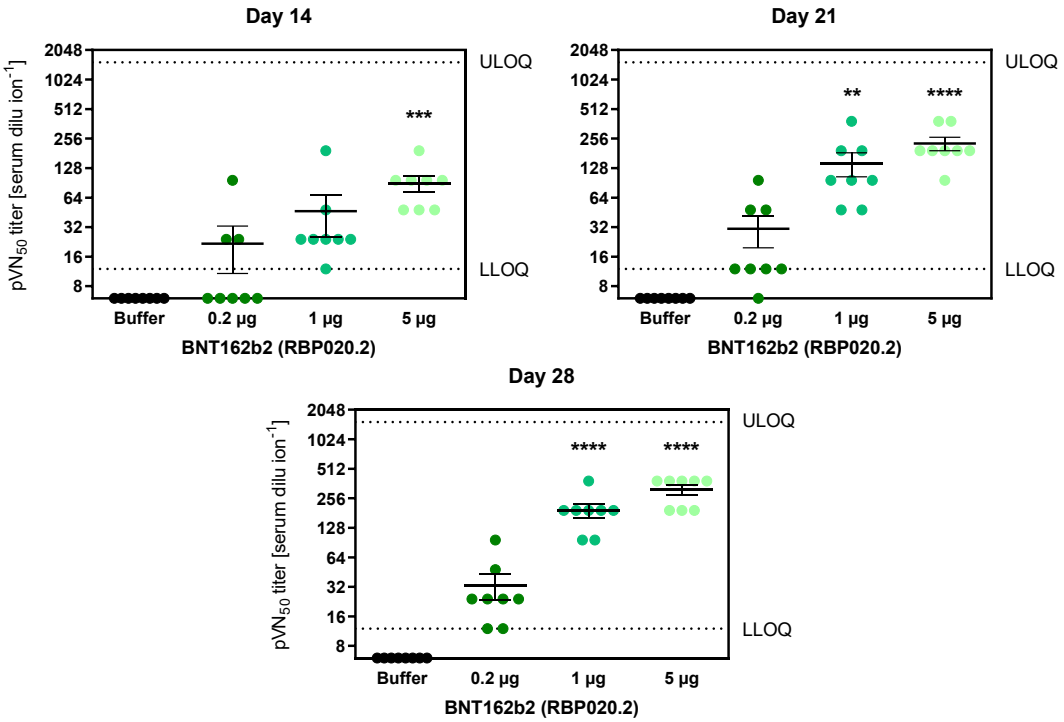

Figure 12: Titers of neutralizing antibodies on days 14, 21, and 28

Serum samples were collected on days 14, 21, and 28 after immunizations and titers of virus-neutralizing ant bodies were determined by pseudovirus-based neutralization test (pVNT). Individual VNT titers are shown by dots; group mean values are indicated by horizontal bars (±SEM, standard error of the mean). ULOQ: Upper limit of quantification, LLOQ: Lower limit of quantification. ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$.

5.4 ELISpot Analysis

Mice were euthanized on day 28 and splenocytes were isolated to assess T-cell responses by ELISpot analysis. Splenocytes were stimulated with S1- and RBD-specific overlapping peptide pools (Table 5) and IFN-γ secretion was detected. Statistical significance was assessed by one-way ANOVA with Dunnett's multiple comparison post-test. Control measurements were performed using an irrelevant peptide pool, medium only or Concanavalin A (ConA, for exemplary controls for the assay performed with frozen splenocytes see Appendix 3: Controls for ELISpot Analysis, Figure 21).

Stimulation of fresh splenocytes with an S protein- or RBD-specific overlapping peptide pool induced IFN-γ responses in T cells of immunized animals (Figure 13). After stimulation with either the S protein-specific or RBD peptide pool, splenocytes of the groups treated with modRNA displayed significantly higher spot numbers than buffer

control splenocytes (for S protein: p = 0.0001 for 0.2 µg, p < 0.0001 for 1 µg; RBD: p = 0.0094 for 0.2 µg and p < 0.0001 for 1 µg).

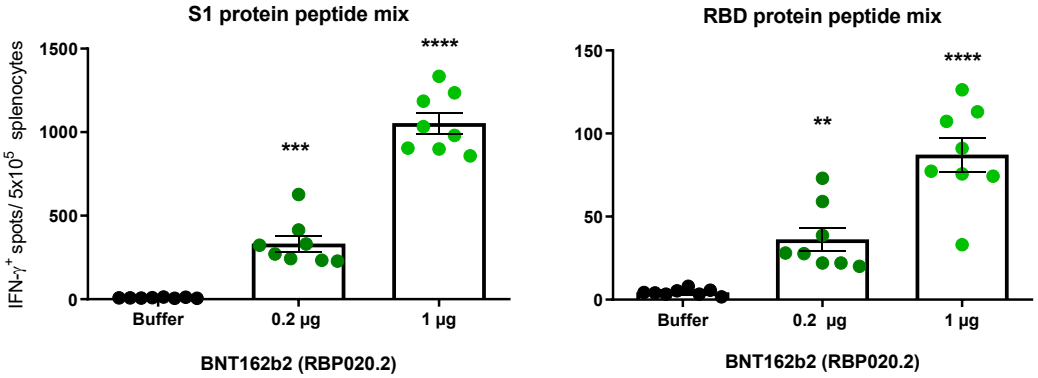

Figure 13: ELISpot analysis using fresh splenocytes on day 28

ELISpot assay was performed using splenocytes isolated on day 28 after immunization. Splenocytes were stimulated with S protein- or RBD-specific overlapping peptide pools and IFN-γ secretion was measured to assess T-cell responses. Individual spot counts are shown by dots; group mean values are indicated by bars (±SEM). ** p ≤ 0.01, **** p < 0.0001. Note that for the 5 µg group, a miscalculated cell number was used in the assay, therefore this group is not included in the graph.

In the assay with fresh splenocytes, a miscalculation of cells in the group immunized with 5 µg modRNA occurred. Therefore, a second ELISpot run was included with frozen splenocytes.

Stimulation of frozen splenocytes with an S protein- or RBD-specific overlapping peptide pool induced IFN-γ responses in T cells of immunized animals (Figure 14). Frozen splenocytes of the groups treated with modRNA displayed significantly higher spot numbers than buffer control splenocytes (p = 0.0087 for 0.2 µg, p < 0.0001 for 1 µg and 5 µg) after stimulation with the S protein-specific peptide pool. Stimulation with the RBD-specific peptide pool induced significantly higher spot numbers in the groups treated with 1 µg and 5 µg modRNA compared to the buffer control group (p = 0.0001 for 1 µg, p = 0.0015 for 5 µg).

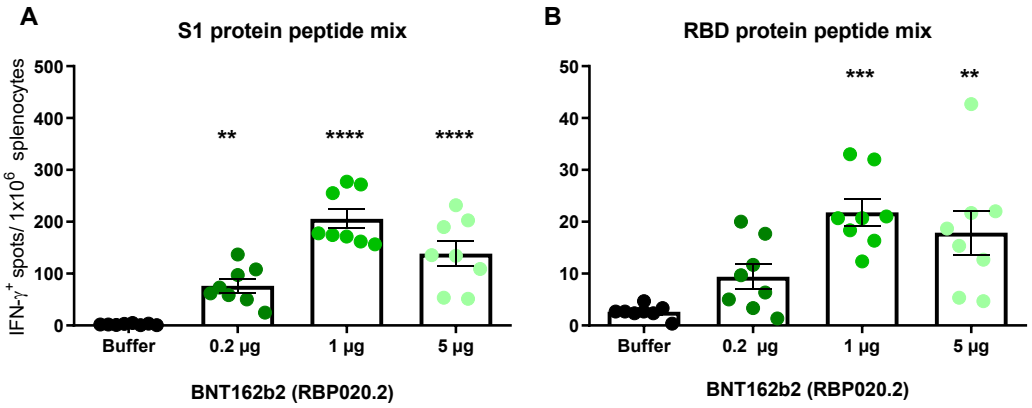

Figure 14: ELISpot analysis using frozen splenocytes on day 28

ELISpot assay was performed using previously frozen splenocytes isolated on day 28 after immunization. Splenocytes were stimulated with S protein- or RBD-specific overlapping peptide pools and IFN-γ secretion was measured to assess T-cell responses. Individual spot counts are shown by dots; group mean values are indicated by bars (±SEM). ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p < 0.0001$.

To identify the responding T-cell subtype, an additional ELISpot analysis was performed after separation of fresh $CD4^+$ and $CD8^+$ cells by MACS isolation using splenocytes isolated from the group treated with 5 µg RNA. Statistical significance was assessed by one-way ANOVA with Dunnett's multiple comparison post-test comparing cells stimulated with RBD- and S protein-specific peptide pools to cells stimulated with an irrelevant AH-1-specific peptide pool.

After stimulation with an S protein-specific peptide pool, but not after stimulation with irrelevant AH-1, both $CD4^+$ and $CD8^+$ cells displayed IFN-γ responses (Figure 15). The differences between cells stimulated with the S protein-specific peptide pool and the cells stimulated with the AH-1-specific peptide pool were statistically significant ($p < 0.0001$ for $CD4^+$ and $CD8^+$ cells). No significant increase in spot numbers was detected in $CD4^+$ and $CD8^+$ cells after stimulation with an RBD-specific peptide pool.

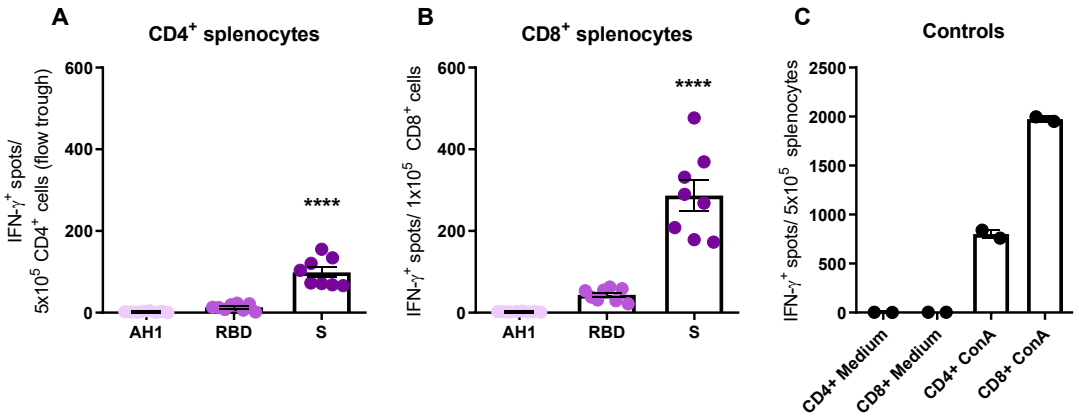

Figure 15: ELISpot analysis using splenocytes of 5 µg BNT162b2 (RBP020.2) immunized mice on day 28 after MACS cell separation

ELISpot assay was performed using splenocytes isolated on day 28 after immunization from group 4 after magnetic cell separation MACS. $CD4^+$ splenocytes (A) or $CD8^+$ splenocytes (B) were stimulated with an RBD- or S protein-specific overlapping peptide pool and IFN-γ secretion was measured to assess T-cell responses. (C) Splenocytes were stimulated with an irrelevant peptide or with medium alone or Concanavalin A. IFN-γ secretion was measured to assess T-cell responses. Mean values ±SEM are shown. **** $p < 0.0001$.

5.5 Luminex Assay

Cytokine concentrations in supernatants of re-stimulated splenocytes were determined using a bead-based, 11-plex T_H1/T_H2 mouse ProcartaPlex immunoassay (Table 8).

Table 8: Chemokines and cytokines included for multiplex measurement

T-cell population	Analytes
T_H1	IFN-γ, GM-CSF, TNF-α, IL-1β, IL-6, IL-12p70, IL-18
T_H2	IL-4, IL-5, IL-13
T_{eff}	IL-2

For cytokine analysis, frozen splenocytes from immunized animals were stimulated with either medium, PMA and ionomycin, or the S- or RBD-overlapping peptide mix.

Immunization with BNT162b2 induced an increased level of T_H1-specific and proinflammatory analytes. Stimulation of splenocytes with 0.1 µg/mL per peptide (total peptide concentration = 31.5 µg/mL) of the S-specific overlapping peptide pool induced a stronger increase in cytokine concentrations than 0.66 µg/mL per peptide (total peptide concentration = 31.5 µg/mL) of the RBD-specific overlapping peptide pool.

Several values were below the lower level of quantification. Therefore, statistical analysis was assessed by mixed-effects analysis/Sidak's comparison. Taking the background of the buffer group, medium control signal into account, a stimulation of

IFN-γ, TNF-α, GM-CSF, IL-6, IL-18, and IL-2 was observed for the groups immunized with 1 µg BNT162b2 (Figure 16). Due to the missing values, statistical significance was not reached or could in some cases not be calculated for the cytokines shown in Figure 16 , even though a clear trend was observed. Therefore, statistical significance is not depicted, but only shown in Appendix 6: Statistical Analysis.

A more detailed summary of the results including PMA controls is shown in Appendix 4: Summary of Luminex Assay.

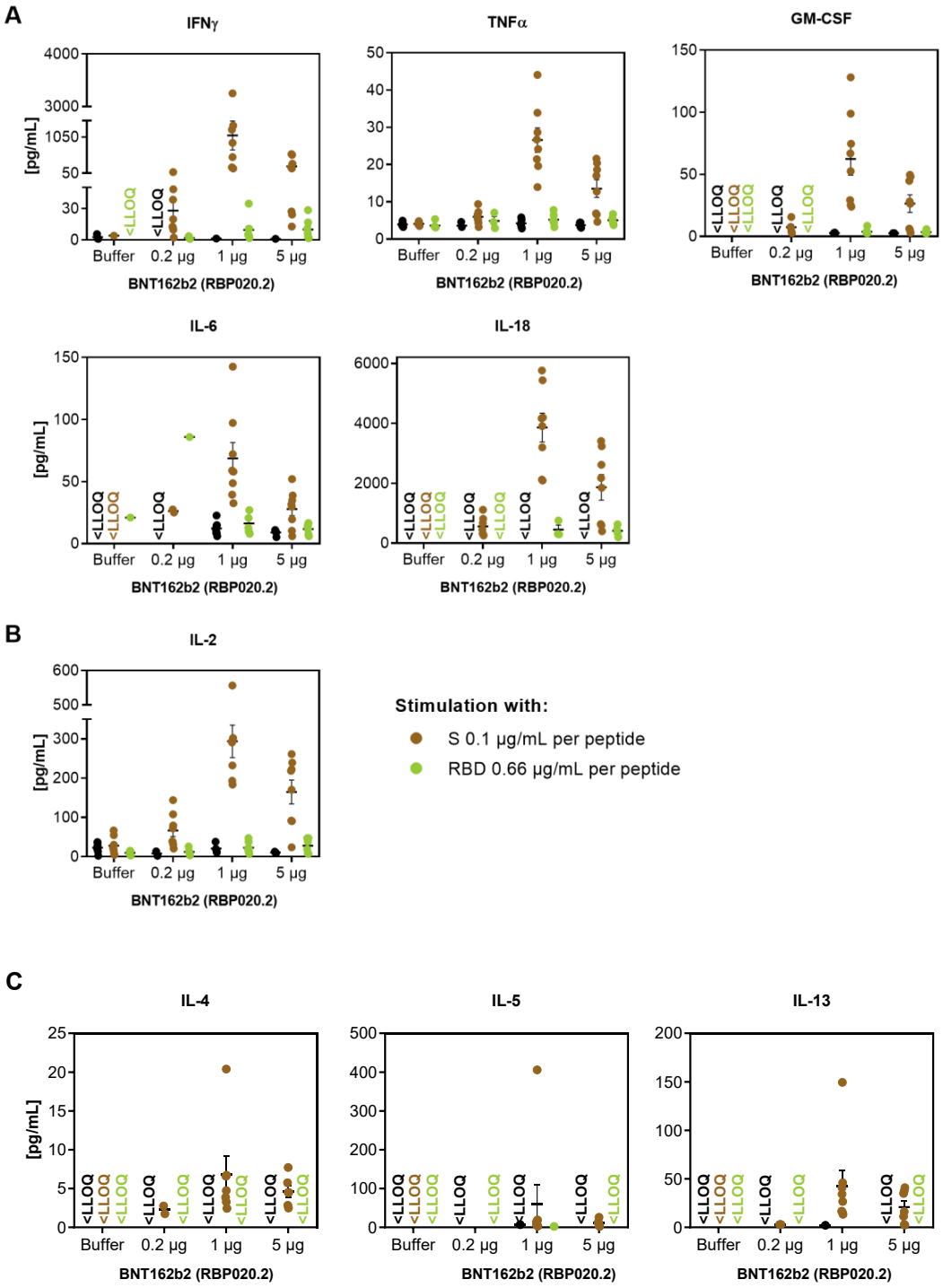

Figure 16: Cytokine concentrations in supernatants of re-stimulated splenocytes 28 days after immunization

Luminex assay was performed using frozen splenocytes isolated on day 28 after RNA injection to assess concentrations of the indicated chemokines/cytokines. After 48 h of stimulation with S- or RBD-overlapping peptide mix, supernatant was collected and secretion of different A) T_H1-specific and proinflammatory, B) T_{eff}-specific and

C) T_H2 cytokines was determined. Values for individual animals are shown by dots. Although all animals within the groups were tested, several values were excluded as they were below the lower level of quantification (LLOQ) and out of standard range. If all values within one group were <LLOQ, this is marked in the graph. Mean values ±SEM are shown.

5.6 Intracellular Cytokine Staining

ICS was performed after stimulation of splenocytes with an overlapping peptide pool of the S protein or controls (Figure 17). Statistical significance was assessed by one-way ANOVA with Dunnett' multiple compari on po t te t

Due to a miscalculation of cells in the group immunized with 5 µg modRNA, ICS results are only shown for buffer control and the groups treated with 0.2 µg and 1 µg BNT162b2.

A peptide-specific stimulation was observed for specific cytokines. The fraction of IFN-γ-expressing $CD4^+$ T cells was significantly higher for animals immunized with 0.2 µg and 1 µg BNT162c2 ($p = 0.0002$ for 0.2 µg, $p < 0.0001$ for 1 µg, Figure 17A) than for buffer control animals. No statistically significant increase was observed for IL-4 after BNT162c2 treatment in comparison to buffer control (Figure 17B). The fraction of TNF-α-expressing $CD4^+$ T cells was significantly higher for animals immunized with 1 µg modRNA ($p < 0.0001$, Figure 17C) than for animals treated with buffer control. For IL-2 expression, the $CD4^+$ T-cell fractions were significantly higher in both treatment groups than in the buffer control group ($p = 0.0015$ for 0.2 µg, $p = 0.0001$ for 1 µg, Figure 17D).

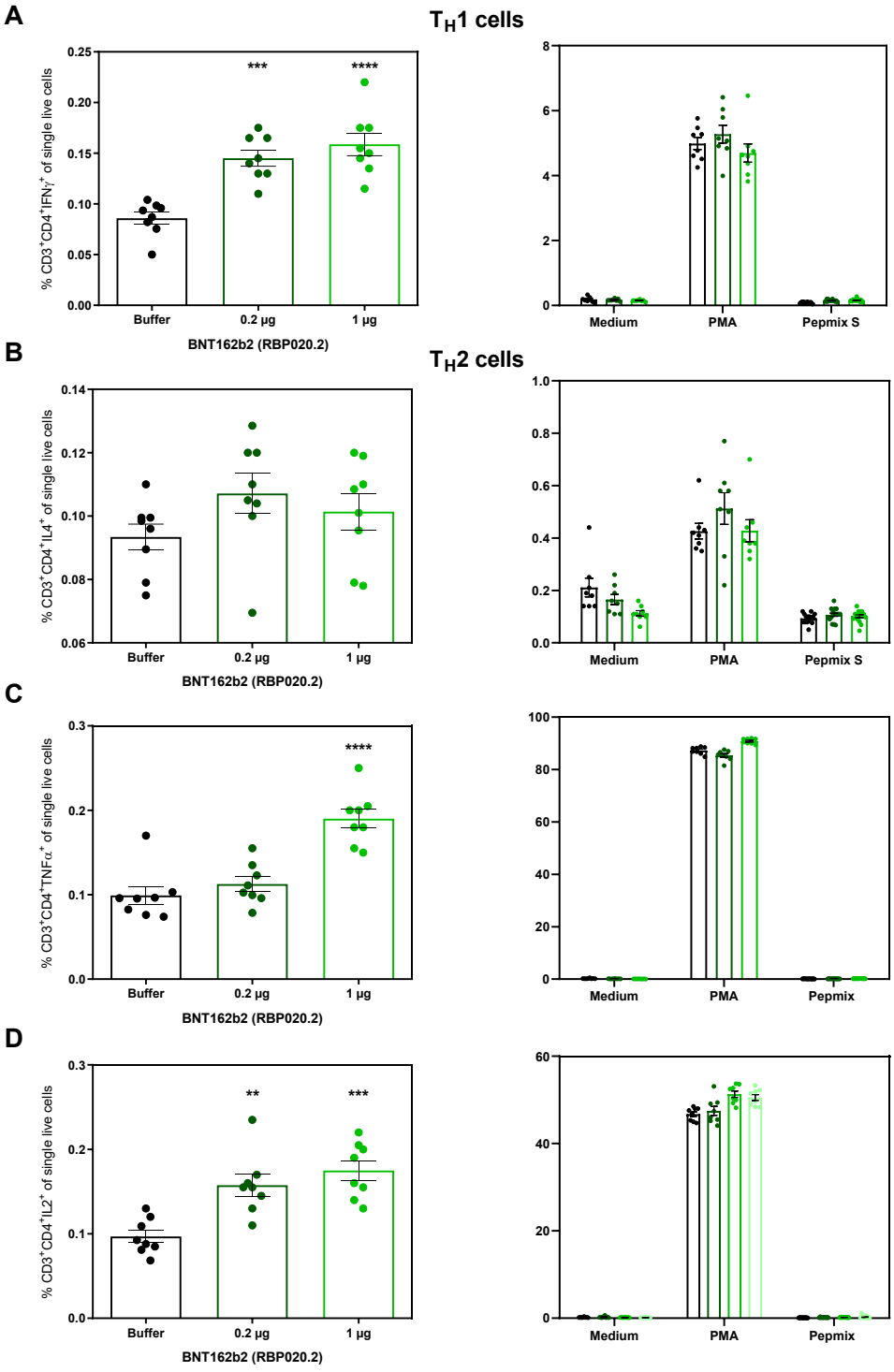

Figure 17: $CD4^+$ T cell intracellular cytokine staining 28 days after immunization

On day 28 after RNA injection, isolated splenocytes were stimulated with either buffer, PMA or an S protein-overlapping peptide mix (Pepmix) to assess the detailed T-cell response via flow cytometry. The intracellular cytokine expression of $CD4^+$ T cells expressing (A) IFN-γ, (B) IL-4, (C) TNF-α, or (D) IL-2 was analyzed. The left

graph in each subfigure shows the fraction after peptide stimulation (measured in duplicates), the right graph shows each single value for all stimulation conditions (buffer and PMA stimulation was measured in single replicates, the Pepmix is the same data as in the left graph but with all single values). Mean values ±SEM are shown. ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

For $CD8^+$ T cells, a statistically significant induction of IFN-γ, TNF-α, and IL-2 was detectable after peptide stimulation in the groups immunized with 0.2 µg and 1 µg RNA compared to buffer control (IFN-γ: $p = 0.0002$ for 0.2 µg, $p < 0.0001$ for 1 µg; TNF-α: $p = 0.0013$ for 0.2 µg, $p < 0.0001$ for 1 µg; IL-2: $p = 0.0003$ for 0.2 µg, $p < 0.0001$ for 1 µg; Figure 18A, B, and C).

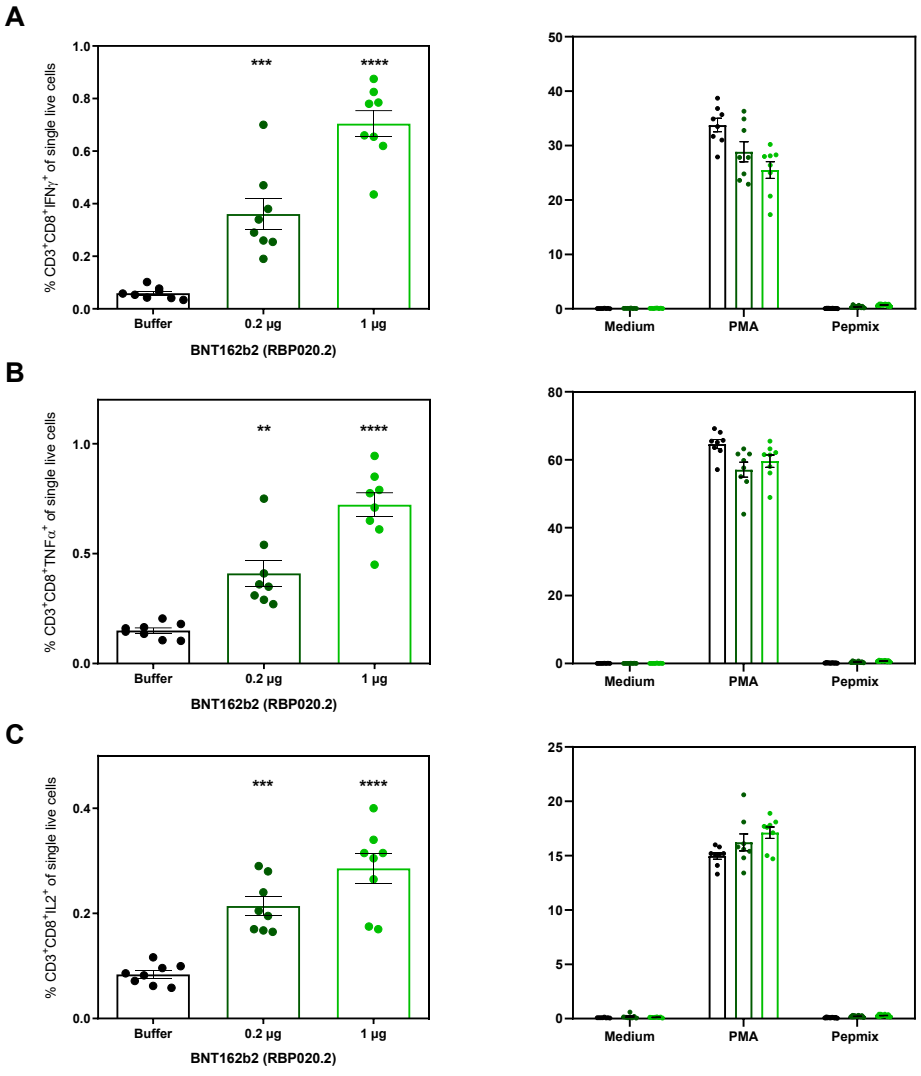

Figure 18: $CD8^+$ T cell intracellular cytokine staining 28 days after immunization

On day 28 after RNA injection, isolated splenocytes were stimulated with either buffer, PMA or an S protein-overlapping peptide mix (Pepmix) to assess the detailed T-cell response via flow cytometry. The intracellular

cytokine expression of $CD8^+$ T cells expressing (A) IFN-γ, (B) TNF-α, or (C) IL-2 was analyzed. The left graph in each subfigure shows the fraction after peptide stimulation (measured in duplicates), the right graph shows each single value for all stimulation conditions (buffer and PMA stimulation was measured in single replicates, the Pepmix is the same data as in the left graph but with all single values). Mean values ±SEM are shown. ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

5.7 Animal Monitoring

The animals' body weight as well as observations regarding fur appearance and injection site reactions are shown in Figure 19 and Figure 20.

The group mean body weights of animals treated with RNA displayed a development comparable to the buffer control group (Figure 19). Over the course of the study, a slight increase in body weights was observed for all groups.

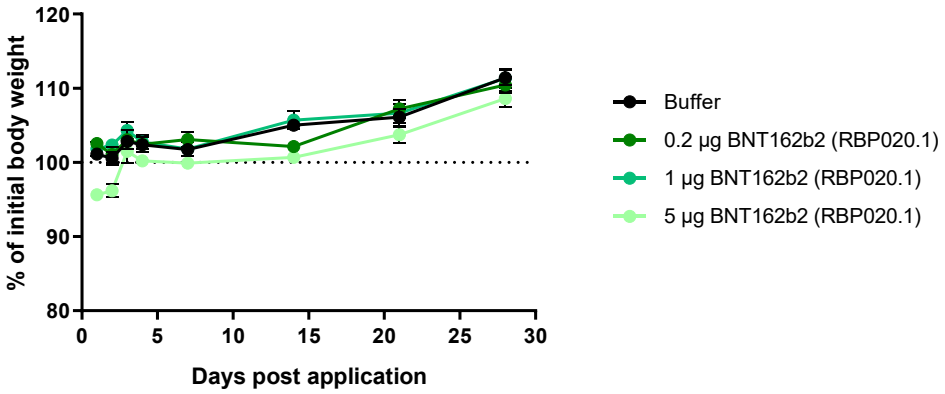

Figure 19: Body weights of experimental mice during study

Experimental animals were weighed at study start and at indicated days, and the change in body weight was calculated as a percentage of the initial weight of the individual mouse. Group mean values (±SEM) are shown.

No changes in fur appearance (i.e., fur defects, neglected grooming, ruffled) were observed in animals treated with BNT162b2 (Figure 20A).

Slight to distinct observations at the injected muscle (i.e., edema formation seen as swollen muscle without flinching in reaction to touch, indicating absence of pain) in comparison to the non-injected hind leg were made in animals treated with 1 µg and 5 µg of BNT162b2 (Figure 20B). By day 2 (1 µg group) or latest by day 4 (5 µg group), the injection site reactions had fully resolved.

Additional animal monitoring details are shown in Appendix 1: Animal Monitoring - Observations.

A

Treatment	Mouse ID	dpi
		1
Buffer	BIO-IJ26	0
	BIO-IJ27	0
	BIO-IJ28	0
	BIO-IJ29	0
	BIO-IJ30	0
	BIO-IJ31	0
	BIO-IJ32	0
	BIO-IJ33	0
0.2 µg BNT162b2 (RBP020.2)	BIO-IJ34	0
	BIO-IJ35	0
	BIO-IJ36	0
	BIO-IJ37	0
	BIO-IJ38	0
	BIO-IJ39	0
	BIO-IJ40	0
	BIO-IJ41	0
1 µg BNT162b2 (RBP020.2)	BIO-IJ42	0
	BIO-IJ43	0
	BIO-IJ44	0
	BIO-IJ45	0
	BIO-IJ46	0
	BIO-IJ47	0
	BIO-IJ48	0
	BIO-IJ49	0
5 µg BNT162b2 (RBP020.2)	BIO-IJ50	0
	BIO-IJ51	0
	BIO-IJ52	0
	BIO-IJ53	0
	BIO-IJ54	0
	BIO-IJ55	0
	BIO-IJ56	0
	BIO-IJ57	0

B

Treatment	Mouse ID	Days post application			
		1	2	3	4
Buffer	BIO-IJ26	0	0	0	0
	BIO-IJ27	0	0	0	0
	BIO-IJ28	0	0	0	0
	BIO-IJ29	0	0	0	0
	BIO-IJ30	0	0	0	0
	BIO-IJ31	0	0	0	0
	BIO-IJ32	0	0	0	0
	BIO-IJ33	0	0	0	0
0.2 µg BNT162b2 (RBP020.2)	BIO-IJ34	0	0	0	0
	BIO-IJ35	0	0	0	0
	BIO-IJ36	0	0	0	0
	BIO-IJ37	0	0	0	0
	BIO-IJ38	0	0	0	0
	BIO-IJ39	0	0	0	0
	BIO-IJ40	0	0	0	0
	BIO-IJ41	0	0	0	0
1 µg BNT162b2 (RBP020.2)	BIO-IJ42	0	0	0	0
	BIO-IJ43	+	0	0	0
	BIO-IJ44	+	0	0	0
	BIO-IJ45	+	0	0	0
	BIO-IJ46	+	0	0	0
	BIO-IJ47	+	0	0	0
	BIO-IJ48	+	0	0	0
	BIO-IJ49	+	0	0	0
5 µg BNT162b2 (RBP020.2)	BIO-IJ50	+++	++	+	0
	BIO-IJ51	++	++	+	0
	BIO-IJ52	++	++	+	0
	BIO-IJ53	++	+	0	0
	BIO-IJ54	++	++	+	0
	BIO-IJ55	+++	++	+	0
	BIO-IJ56	++	+	0	0
	BIO-IJ57	++	+	0	0

Figure 20: Summary of observations made during study's concomitant animal monitoring

Shown are deviations to normal appearance in (A) fur condition and (B) observations at the injection site (edema formation) of each mouse. Severity of observations is graded with 0, none; +, slight; ++, moderate; and +++, distinct.

6 CONCLUSION

Treatment with all tested BNT162b2 doses, namely 0.2, 1 and 5 µg, induced a strong immune response across the observation period of 28 days after vaccination with a safe profile in terms of animal monitoring.

Total IgG ELISA showed that the construct is immunogenic and induced a strong, dose-dependent generation of antibodies against the S1 antigen and the receptor-binding domain. First detection of IgG antibodies was possible 7 days after immunization for all animals throughout the groups with an increase of total antibody amount until day 28. At day 28 after immunization, vaccine-elicited IgG had a strong binding affinity for S1 (geometric mean K_D = 12 nM) and the RBD (geometric mean K_D = 0.99 nM), both had low off-rates.

Profiling the IgG subtypes, a balanced IgG2a/IgG1 response was detected for the two higher doses, while the low dose induced a response with higher IgG1 than IgG2 levels. In pVNT analysis, starting 14 days after immunization, a development of functional neutralizing antibodies was shown for all animals and the titers increased until the final study day. The summary of antibody titers at day 28 is as follows:

	BNT162b2 0.2 µg	**BNT162b2 1 µg**	**BNT162b2 5 µg**
Anti-S1 protein total IgG [µg/mL]	73.0 ± 10.4	205.9 ± 21.0	392.7 ± 28.9
Anti-RBD protein total IgG [µg/mL]	83.1 ± 12.3	241.7 ± 17.2	448.6 ± 28.6
pVN_{50} titer [reciprocal dilution]	33.0 ± 9.8	192.0 ± 31.4	312.0 ±35.1

The ELISpot assay confirmed a strong T-cell activation with the dose of 1 µg resulting in the strongest reactivity. An additional ELISpot with $CD8^+$- and $CD4^+$-separated T cells showed both a reactive $CD8^+$ and $CD4^+$ T-cell response. In Luminex analysis, chemokines and cytokine production after peptide stimulation was confirmed for the group dosed with 1 µg for analytes that indicate a T_H1-driven and proinflammatory immune response in line with the ELISpot. Similarly, reactive IFN-γ-, TNF-α-, and IL-2-secreting $CD4^+$ as well as $CD8^+$ T cells were detected after peptide stimulation in ICS. Taken together, the cellular analysis revealed that in addition to a cytotoxic $CD8^+$ T-cell response, a T_H1-specific response was activated after peptide stimulation.

In summary, the vaccine candidate was highly immunogenic and induced high IgG and neutralizing antibody titers against the antigen as well as a desired T_H1-driven T-cell response including a strong cytotoxic T-cell response. Therefore, BNT162b2 is a promising candidate for further testing in clinical trial.

7 DOCUMENT HISTORY

Reasons for changes compared to previous version:

Minor editorial changes, such as the correction of typing errors, are not specifically listed.

Sections	Version 01	Version 02	Reason for change
1 2.4 3.3 4.5.6 5.2 6	-	Further experimental information added	Added SPR measurements of binding affinities of BNT162b2 vaccine-induced SARS-CoV-2-specific antibodies toward recombinant SARS-CoV-2 S and RBD proteins.
4.4	-	Further equipment and software information added	Equipment table was added and software table was updated.

Sections	Version 02	Version 03	Reason for change
List of Abbreviations	-	Update of list	Additional abbreviations included.
2.4 4.5.5 5.1.1	-	Further experimental information added	Reciprocal endpoint serum titer added for day 14 and day 28 serum samples.
4.5.10 5.5 9	-	Luminex	The used peptide concentration was corrected and the CoA of the ProcartaPlex was included.
4.5.11	-	ICS	The used peptide concentration was corrected.
5.2	-	SPR	Table corrected.

Sections	Version 03	Version 04	Reason for change
4.5.3.1	-	Information for 3 doses of BNT162b2 modified	Doses corrected

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9 APPENDIX

Appendix 1: Animal Monitoring - Observations

Table 9: Parameters for experimental animal monitoring (single mouse assessment)

The table is separated in immediate euthanasia criteria (end of experiment) and criteria which solitarily observed do not lead to an immediate termination, but result in shorter monitoring frequency (re-assessment).

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate cumulation**	**Immediate euthanasia criteria**
1	Bodyweight [b]. Take into account Body Conditioning Score (BCS) [c]	Body weight loss > 5 – 10% or BCS transition 3 → 2	Bodyweight loss > 15 - 20% or BCS 2
2	Activity	Moderate deviation from normal or unusual behavior (e.g., limited, reduced, or hyperactive movements)	Immobility, very slow movements (high grade of lethargy), self-isolation
3	Appearance (condition) of fur & eyes	Fur defects/ grooming malfunction (reduced or exaggerated grooming). Moderate orbital tightening.	Distinct scruffy fur, strongly neglected grooming. Eyes lids narrowed, eyes closed and sticky.
4	Body cavities & body fluids	slight - moderate damp & sticky cavities	Clinical signs of disease (diarrhea, distinct sticky)
5	Body temperature & blood circulation ears	-	Animal's body temperature low, ears appear white, and hardly noticeable blood vessels
6	Posture	Moderate deviation of normal physiological posture i.e., short pause in hunched posture	Abnormal posture, hunched, abnormally stretched (belly touches ground) or cramps
7	Reaction to stimulus [d]	Delayed reaction to unconditioned stimulus, moderate deviation from normal behavior (e.g., slight - moderate apathy)	Abnormal (distinct delayed reaction to unconditioned stimulus). Winding and enduring sound utterance ("pain"), aggressiveness at touch
8	Automutilation	-	Burden for the animal noticeable i.e., missing extremities, continuous nibbling, biting and gnawing, open wounds

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate cumulation**	**Immediate euthanasia criteria**
9	Bites (tail, vibrissae, reproductive organs), other wounds	Open and bleeding wounds (take care of wounds and separate animal)	Burden for the animal noticeable i.e., inflamed wounds
10	Respiration frequency	Moderate deviation of spontaneous breathing (normal respiration frequency)	High frequency, any sign of dyspnea, gasping, flat stretched posture in combination with strongly retracting flanks
11	Motor function	Weak, loose grip (cage grid)	Staggering, circular movement, missing grasp
12	Other abnormalities [e]	-	-

a Categories: NAD, no abnormality detected; +, slight; ++, moderate; +++, distinct.

b Calculate ratio bodyweight start of experiment/ bodyweight monitoring day.

c According to Ullman-Culleré and Foltz 1999.

d Unconditioned = Stimulus to force a reaction e.g., normal background noise, tapping the cage, and normal handling procedure e.g., tilt and turns of the cage.

e Description of abnormality (or abnormalities) on monitoring sheet.

Table 10: Record of body weights of experimental mice during study

						Bodyweight (grams)								
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21	Day 28
SBIO-15164	BIO-LJ26	BALB/cJRj	f	28.01 20	1	21.4	21.6	21.6	22 0	21.9	21.7	22.1	22.2	24.4
SBIO-15164	BIO-LJ27	BALB/cJRj	f	28.01 20	1	22.0	22.4	21.6	22 2	23.9	22.2	23.1	22.9	24.2
SBIO-15164	BIO-LJ28	BALB/cJRj	f	28.01 20	1	19.9	20.8	20.7	21.1	20.7	20.3	21.3	20.9	22.7
SBIO-15164	BIO-LJ29	BALB/cJRj	f	28.01 20	1	22.3	22.8	23.1	23.4	22.3	22.7	23.2	24.4	24.2
SBIO-15165	BIO-LJ30	BALB/cJRj	f	28.01 20	1	21.6	21.2	20.9	21 5	21.4	21.2	23.4	22.4	23.9
SBIO-15165	BIO-LJ31	BALB/cJRj	f	28.01 20	1	20.8	20.8	20.8	21 2	21.2	21.7	21.9	23.4	24.0
SBIO-15165	BIO-LJ32	BALB/cJRj	f	28.01 20	1	21.8	22.1	22.2	22 8	22.1	22.6	22.8	23.4	24.2
SBIO-15165	BIO-LJ33	BALB/cJRj	f	28.01 20	1	22.1	22.1	21.9	22 5	22.5	22.5	22.7	22.8	23.8
SBIO-15166	BIO-LJ34	BALB/cJRj	f	28.01 20	2	20.1	20.6	19.9	20 3	20.0	20.6	20.5	21.6	21.8
SBIO-15166	BIO-LJ35	BALB/cJRj	f	28.01 20	2	20.6	20.6	19.8	19.7	20.1	20.1	20.3	20.7	21.8
SBIO-15166	BIO-LJ36	BALB/cJRj	f	28.01 20	2	19.7	20.7	20.3	20 5	20.0	20.3	20.1	20.7	21.3
SBIO-15166	BIO-LJ37	BALB/cJRj	f	28.01 20	2	20.3	20.2	20.2	20 5	20.7	21.9	21.0	22.5	22.8
SBIO-15167	BIO-LJ38	BALB/cJRj	f	28.01 20	2	21.2	21.7	21.6	22.7	21.8	21.6	21.9	23.3	24.1
SBIO-15167	BIO-LJ39	BALB/cJRj	f	28.01 20	2	21.2	21.7	21.3	22.1	22.3	21.7	21.8	23.1	23.5
SBIO-15167	BIO-LJ40	BALB/cJRj	f	28.01 20	2	19.6	20.8	20.9	20 8	20.7	20.6	20.6	21.4	22.6
SBIO-15167	BIO-LJ41	BALB/cJRj	f	28.01 20	2	21.0	21.5	21.4	22.1	22.1	21.9	21.0	22.2	22.9
SBIO-15168	BIO-LJ42	BALB/cJRj	f	28.01 20	3	19.7	19.7	19.7	19 5	19.6	19.6	19.9	21.1	20.8
SBIO-15168	BIO-LJ43	BALB/cJRj	f	28.01 20	3	21.2	22.5	22.0	22 6	23.1	22.2	22.7	23.3	23.5
SBIO-15168	BIO-LJ44	BALB/cJRj	f	28.01 20	3	21.7	21.5	21.8	22 5	22.4	20.9	23.6	21.9	24.4
SBIO-15168	BIO-LJ45	BALB/cJRj	f	28.01 20	3	20.8	21.1	21.1	21 8	21.4	21.6	22.6	23.5	23.5
SBIO-15169	BIO-LJ46	BALB/cJRj	f	28.01 20	3	20.5	20.6	21.0	20 8	20.7	20.9	21.4	22.0	22.6
SBIO-15169	BIO-LJ47	BALB/cJRj	f	28.01 20	3	20.2	20.4	20.5	21 6	20.6	20.5	20.7	21.2	22.8
SBIO-15169	BIO-LJ48	BALB/cJRj	f	28.01 20	3	19.8	20.9	20.9	21 5	20.1	20.7	21.9	20.9	23.3
SBIO-15169	BIO-LJ49	BALB/cJRj	f	28.01 20	3	20.5	20.7	21.2	21 2	21.1	20.9	21.0	21.4	22.2
SBIO-15170	BIO-LJ50	BALB/cJRj	f	28.01 20	4	22.6	22.1	22.9	23 6	23.6	22.7	23.2	24.5	24.9
SBIO-15170	BIO-LJ51	BALB/cJRj	f	28.01 20	4	22.4	21.5	21.5	22.1	22.3	22.1	23.0	22.8	23.9
SBIO-15170	BIO-LJ52	BALB/cJRj	f	28.01 20	4	21.2	20.9	20.3	20 8	21.2	21.6	21.0	21.7	23.2
SBIO-15170	BIO-LJ53	BALB/cJRj	f	28.01 20	4	21.8	20.5	21.2	21 8	22.1	22.1	22.0	22.9	25.1
SBIO-15171	BIO-LJ54	BALB/cJRj	f	28.01 20	4	20.3	19.7	19.5	20 2	20.1	20.0	20.5	20.8	22
SBIO-15171	BIO-LJ55	BALB/cJRj	f	28.01 20	4	20.5	19.2	19.1	22 9	20	20.8	20.4	20.2	22
SBIO-15171	BIO-LJ56	BALB/cJRj	f	28.01 20	4	22	20.8	21.2	21 8	22.2	22.3	22.1	22.9	23.4
SBIO-15171	BIO-LJ57	BALB/cJRj	f	28.01 20	4	22.8	21.3	21.3	22 9	22.5	21.8	22.6	24.4	24

Table 11: Record of animal monitoring for each mouse during study

						Animal Monitoring - Observations								
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21	Day 28
SBIO-15164	BIO-LJ26	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15164	BIO-LJ27	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15164	BIO-LJ28	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15164	BIO-LJ29	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15165	BIO-LJ30	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15165	BIO-LJ31	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15165	BIO-LJ32	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15165	BIO-LJ33	BALB/cJRj	f	28.01.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15166	BIO-LJ34	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15166	BIO-LJ35	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15166	BIO-LJ36	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15166	BIO-LJ37	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15167	BIO-LJ38	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15167	BIO-LJ39	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15167	BIO-LJ40	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15167	BIO-LJ41	BALB/cJRj	f	28.01.20	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15168	BIO-LJ42	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15168	BIO-LJ43	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15168	BIO-LJ44	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15168	BIO-LJ45	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15169	BIO-LJ46	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15169	BIO-LJ47	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15169	BIO-LJ48	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15169	BIO-LJ49	BALB/cJRj	f	28.01.20	3	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15170	BIO-LJ50	BALB/cJRj	f	28.01.20	4	NAD	12+++	12++	12+	NAD	NAD	NAD	NAD	NAD
SBIO-15170	BIO-LJ51	BALB/cJRj	f	28.01.20	4	NAD	12++	12++	12+	NAD	NAD	NAD	NAD	NAD
SBIO-15170	BIO-LJ52	BALB/cJRj	f	28.01.20	4	NAD	12++	12++	12+	NAD	NAD	NAD	NAD	NAD
SBIO-15170	BIO-LJ53	BALB/cJRj	f	28.01.20	4	NAD	12++	12+	ok	NAD	NAD	NAD	NAD	NAD
SBIO-15171	BIO-LJ54	BALB/cJRj	f	28.01.20	4	NAD	12++	12++	12+	NAD	NAD	NAD	NAD	NAD
SBIO-15171	BIO-LJ55	BALB/cJRj	f	28.01.20	4	NAD	12+++	12++	12+	NAD	NAD	NAD	NAD	NAD
SBIO-15171	BIO-LJ56	BALB/cJRj	f	28.01.20	4	NAD	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15171	BIO-LJ57	BALB/cJRj	f	28.01.20	4	NAD	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD

Appendix 2: Certificates of Analysis

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel.: +49 (0) 6131-90 84-0, Fax: +49 (0) 6131-90 84-390
info@biontech.de

BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.2 (ATM batch modRNAv09)**
Lot/Batch No.:	RNA-RF200321-06
RNA length:	4283 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	19 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Denaturing Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6) (b) (6)

Report of Results_ RBP020.2 (ATM batch modRNAv09); Version 01 1/1

POLYMUN SCIENTIFIC
Immunbiologische Forschung GmbH
Donaustraße 99
A-3400 Klosterneuburg, Austria
Tel.: +43-2243-25060-300
Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.2LNP
Lot: CoVVAC/270320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 09.04.20 (b) (6)

Date: 09.04.20

CarVac # 09

ThermoFisher SCIENTIFIC

Bender MedSystems GmbH
Campus Vienna Biocenter 2
A-1030 Vienna, Austria
www.thermofisher.com

Certificate of analysis

ProcartaPlex

Cat. number: **EPX110-20820-901**
96 tests/11 analytes

Name: **ProcartaPlex Mouse Th1/Th2 Cytokine Panel 11plex**

Lot number: **232634-004**

Expiry date: **2022-04**

Components		Quantity	Lot	Store at
BK20820EX	det. antibody Mix B	1 x 70µl (50x)	202902000	2-8°C
B20820EX	Bead Mix B	1 x 5ml (1x)	202901000	2-8°C
S26088EX	Standard Mix A	2 each	220399-001	2-8°C
DDBMEX/4	Detection Ab Diluent	1 x 3ml	205752000	2-8°C
RBEX/46	Reading Buffer	1 x 40ml	19127887	2-8°C
WBEX/28	10x Wash Buffer	1 x 25ml	19127883	2-8°C
SA-PE	Streptavidin-PE	1 x 5ml	233434-000	2-8°C
UABEX/11	Universal Assay Buffer 1x	1 x 10ml	20018141	2-8°C
SVM104	Black Microplate Lid	1 each		2-8°C
SVM16	Plate Covers	8 each		2-8°C
SVM182	Flat bottom Plate (black)	1 each		2-8°C
SVM183	PCR 8-Tube Strip	2 each		2-8°C

Bead Mix B Lot#202901000

Target Name	Bead Number	Std1 Concentration pg/ml	Standard
GM-CSF	42	9950	Standard Mix A
IFN gamma	38	4800	Standard Mix A
IL-1 beta	19	4350	Standard Mix A
IL-12p70	39	6550	Standard Mix A
IL-13	35	8650	Standard Mix A
IL-18	66	207000	Standard Mix A
IL-2	20	5250	Standard Mix A
IL-4	26	4950	Standard Mix A
IL-5	27	8000	Standard Mix A
IL-6	28	19500	Standard Mix A
TNF alpha	45	11700	Standard Mix A

Standard Mix A Lot#220399-001

Provided below is a table of Standard 1 (Std1) value for each analyte in each tube when prepared according to the "Preparing Standard" procedure of the Manual.

Analyte	Std1 Concentration (pg/ml)	ULOQ / LLOQ (pg/ml) Determined in cell culture medium
Eotaxin (CCL11)	2550	2550 / 0,62
GM-CSF	9950	9950 / 2,43
GRO alpha (CXCL1)	5950	5950 / 1,45
IFN gamma	4800	4800 / 1,17
TNF alpha	11700	11700 / 2,86
IL-10	8400	8400 / 2,05
IL-12p70	6550	1638 / 1,60
IL-13	8650	8650 / 2,11
IL-17A (CTLA-8)	5750	5750 / 1,40
IL-18	207000	51750 / 51
IL-1 beta	4350	4350 / 1,06
IL-2	5250	5250 / 1,28
IL-22	40400	40400 / 9,86
IL-23	34500	34500 / 8,42
IL-27	8350	8350 / 2,04
IL-4	4950	4950 / 1,21
IL-5	8000	8000 / 1,95
IL-6	19500	19500 / 4,76
IL-9	66900	66900 / 16
IP-10 (CXCL10)	2250	2250 / 0,55
MCP-1 (CCL2)	28300	28300 / 6,91
MCP-3 (CCL7)	900	900 / 0,22
MIP-1 alpha (CCL3)	1400	1400 / 0,34
MIP-1 beta (CCL4)	6200	1550 / 1,51
MIP-2 alpha (CXCL2)	3350	838 / 3,27
RANTES (CCL5)	10800	2700 / 2,64

Analytical information:

This product has been tested by Quality Control and passed internal specifications.

Quality control: (b) (6)

For Research Use Only. Not for use in diagnostic procedures. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1-800-955-6288 (US and Canada) or 1-760-603-7200, x2 (all other countries).
For inquiries, contact us at "thermofisher.com/askaquestion"

Appendix 3: Controls for ELISpot Analysis

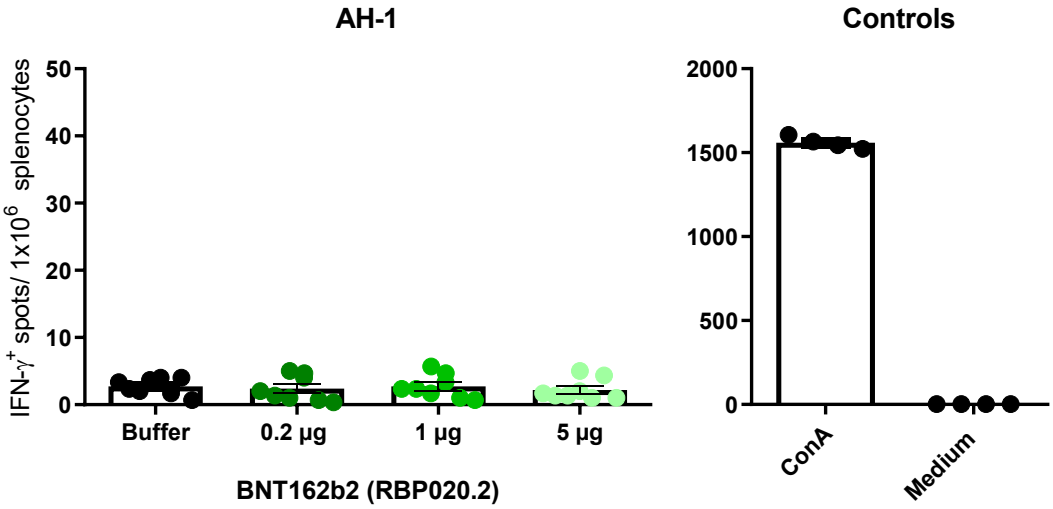

Figure 21: Controls for ELISpot analysis using splenocytes on day 28

ELISpot assay was performed using splenocytes isolated on day 28 after immunization. Splenocytes were stimulated with the irrelevant peptide AH-1 (left), or with Concanavalin A or medium alone (right). IFN-γ secretion was measured to assess T-cell responses. Mean values ±SEM are shown.

Appendix 4: Summary of Luminex Assay Data

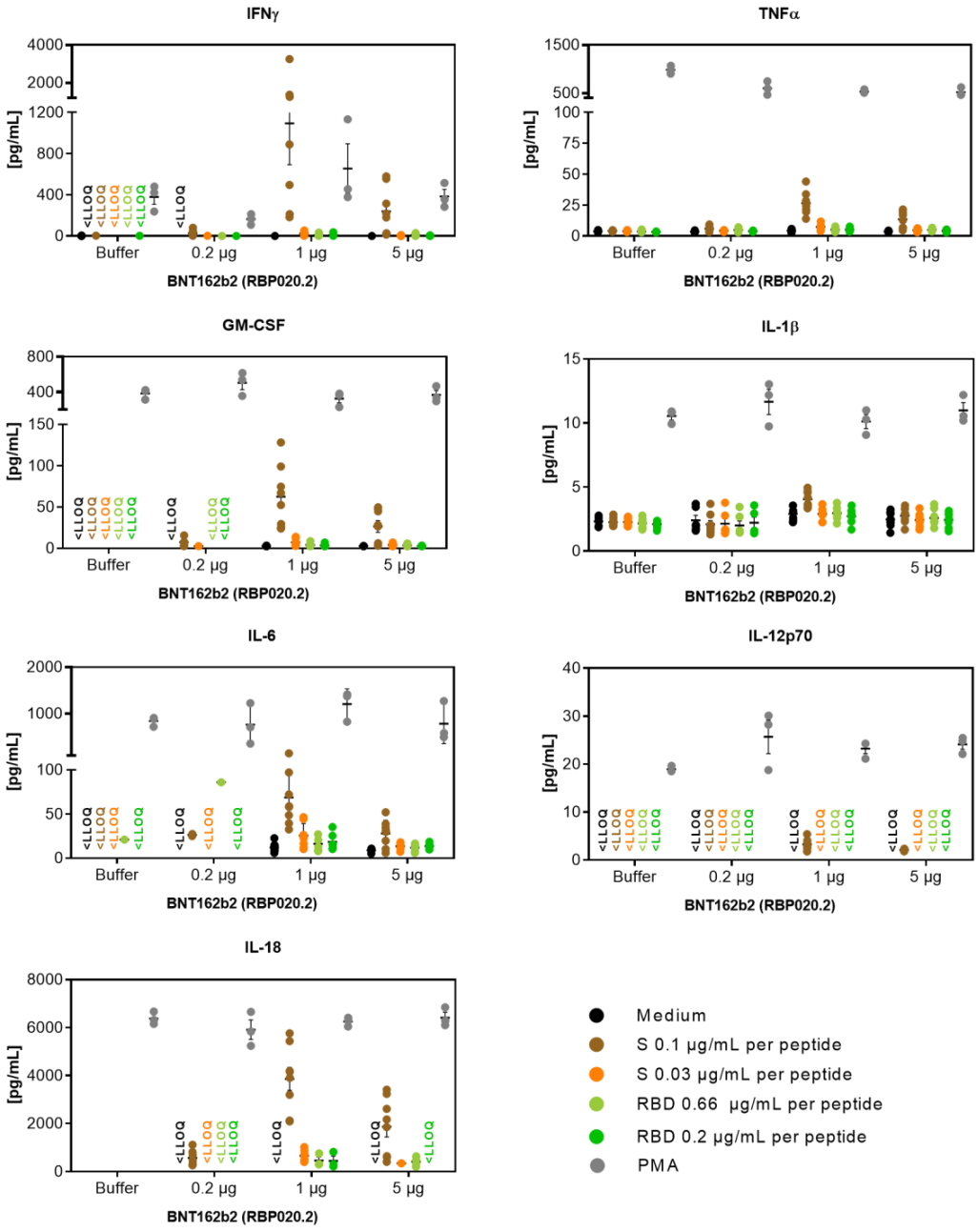

Figure 22: T_H1 and proinflammatory cytokine concentrations in supernatants of re-stimulated splenocytes 28 days after immunization

Luminex assay was performed using frozen mouse splenocytes isolated on day 28 after RNA injection to assess concentrations of the indicated chemokines/cytokines. After 48 h of stimulation with medium, PMA plus ionomycin (PMA), or the S-/RBD-overlapping peptide mix, supernatant was used for the analysis of the secretion of different analytes. Values for individual animals are shown by dots; group mean values are indicated by lines (±SEM). Several values were excluded, as they were below the LLOD or out of the standard range (<LLOQ) or upper the limit of quantification (ULOQ). Therefore, no statistical information was included in the figure (for calculation, see Appendix 6: Statistical Analysis). Medium, S 0.1 µg/mL and RBD 0.66 µg/mL per peptide are shown in Figure 16.

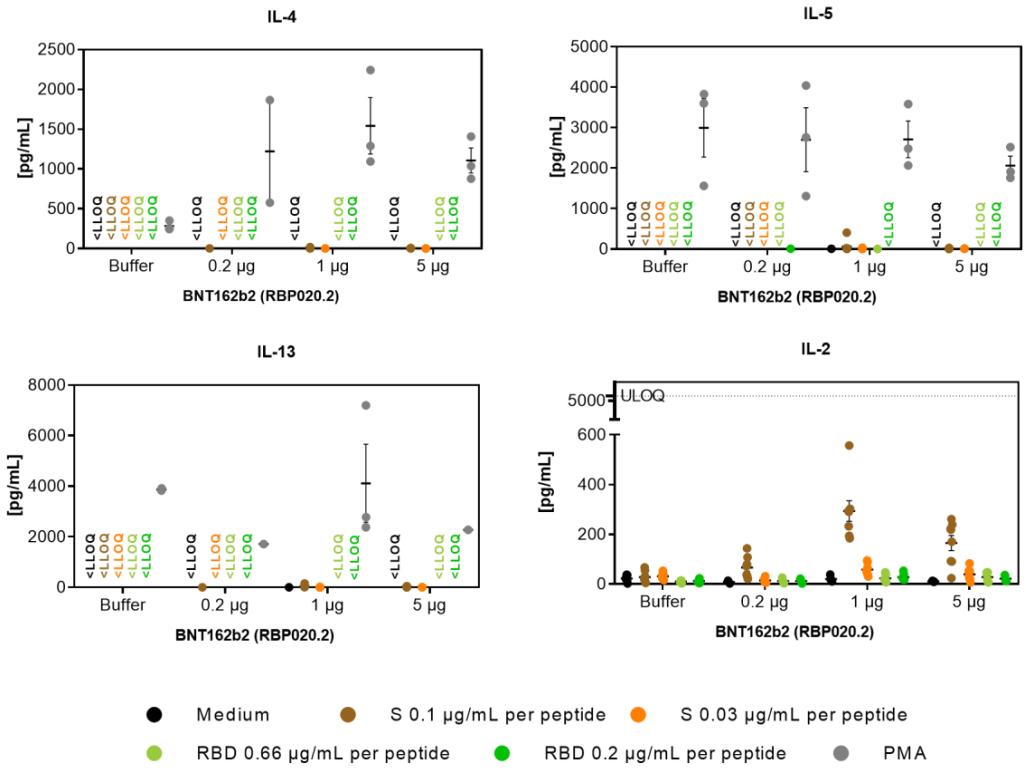

Figure 23: T_H2 cytokine and IL-2 concentrations in supernatants of re-stimulated splenocytes 28 days after immunization

Luminex assay was performed using frozen mouse splenocytes isolated on day 28 after RNA injection to assess concentrations of the indicated chemokines/cytokines. After 48 h of stimulation with medium, PMA plus ionomycin (PMA), or the S-/RBD-overlapping peptide mix, supernatant was used for the analysis of the secretion of different analytes. Values for individual animals are shown by dots; group mean values are indicated by lines (±SEM). The RBD peptide mix was not tested for animals treated with 0.2 µg and 1 µg RNA. Several values were excluded, as they were below the LLOD or out of the standard range (<LLOQ). Therefore, no statistical information was included in the figure (for calculation, see Appendix 6: Statistical Analysis). Medium, S 0.1 µg/mL and RBD 0.66 µg/mL per peptide are shown in Figure 16. Note after PMA stimulation, all IL-2 concentrations were above the upper limit of quantification (ULOQ).

Appendix 5: Detailed ICS Protocol

FACS panel (FACS Celesta)

	Wavelengths	Markers	µL per 50 µL	Clones	Colors	Name	Company	Catalog no.
1	450/50	CD8a	0.25	53-6.7	BV421	Brilliant Violet 421™ anti-mouse CD8a antibody	BioLegend	100753
2	525/50	CD4	0.25	RM4-5	BV510	Brilliant Violet 510™ anti-mouse CD4 antibody	BioLegend	100559
	610/20		no		BV605			
3	710/50	IL-4	0.25	11B11	BV711	Brilliant Violet 711™ anti-mouse IL-4 antibody	BioLegend	504133
4	780/60	CD25	0.25	PC61	BV786	Brilliant Violet 785™ anti-mouse CD25 antibody PC61	BioLegend	102051
5	530/30	TNF-α	0.5	MP6-XT22	Alexa 488	Alexa Fluor® 488 anti-mouse TNF-α ant body, clone MP6-XT22	BioLegend	506313
6	575/25	CD3	0.25	145-2C11	PE	PE Hamster anti-mouse CD3e clone 145-2C11	BD	553064
7	695/40		no		PerCP-Cy5.5			
8	780/60	IFN-γ	0.1	XMG1.2	Pe-Cy7	PE/Cy7 anti-mouse IFN-γ antibody, clone XMG1.2	BioLegend	505826
9	670/30	IL-2	0.5	JES6-5H4	APC	APC anti-mouse IL-2 antibody	BioLegend	503810
10	730/45		no		APC-R700			
11	780/60	dead	0.05-0.03		eFluor780	eBioscience™ Fixable Viability Dye eFluor™ 780	ThermoFisher	65-0865-14

96-well plates:

Plate 1: against S protein

	1	2	3	4	5	6	7	8	9	10	11	12
A	pool*[1]	pool*[1]	pool*[1]	pool*[1]		1-1	1-1	1-1	1-1			
B	pool*[2]	pool*[2]	pool*[2]	pool*[2]		1-2	1-2	1-2	1-2			
C	pool*[3]	pool*[3]	pool*[3]	pool*[3]		1-3	1-3	1-3	1-3			
D						1-4	1-4	1-4	1-4			
E						1-5	1-5	1-5	1-5			
F						1-6	1-6	1-6	1-6			
G						1-7	1-7	1-7	1-7			
H						1-8	1-8	1-8	1-8			

Plate 2: against S protein

	1	2	3	4	5	6	7	8	9	10	11	12
A	2-1	2-1	2-1	2-1	3-1	3-1	3-1	3-1	4-1	4-1	4-1	4-1
B	2-2	2-2	2-2	2-2	3-2	3-2	3-2	3-2	4-2	4-2	4-2	4-2
C	2-3	2-3	2-3	2-3	3-3	3-3	3-3	3-3	4-3	4-3	4-3	4-3
D	2-4	2-4	2-4	2-4	3-4	3-4	3-4	3-4	4-4	4-4	4-4	4-4
E	2-5	2-5	2-5	2-5	3-5	3-5	3-5	3-5	4-5	4-5	4-5	4-5
F	2-6	2-6	2-6	2-6	3-6	3-6	3-6	3-6	4-6	4-6	4-6	4-6
G	2-7	2-7	2-7	2-7	3-7	3-7	3-7	3-7	4-7	4-7	4-7	4-7
H	2-8	2-8	2-8	2-8	3-8	3-8	3-8	3-8	4-8	4-8	4-8	4-8

Plate 3: against RBD

	1	2	4	5	5	6	7	8	9	10	11	12
A	1-1	1-1		2-1	2-1		3-1	3-1		4-1	4-1	
B	1-2	1-2		2-2	2-2		3-2	3-2		4-2	4-2	
C	1-3	1-3		2-3	2-3		3-3	3-3		4-3	4-3	
D	1-4	1-4		2-4	2-4		3-4	3-4		4-4	4-4	
E	1-5	1-5		2-5	2-5		3-5	3-5		4-5	4-5	
F	1-6	1-6		2-6	2-6		3-6	3-6		4-6	4-6	
G	1-7	1-7		2-7	2-7		3-7	3-7		4-7	4-7	
H	1-8	1-8		2-8	2-8		3-8	3-8		4-8	4-8	

	* controls	[1]No Ab	[2]CD3+ L/D	[3]LD + CD3 + CD4 + CD8+ L/D
	Medium only	Positive stimulus	S protein	
			RBD	

Mastermixes for stimulation

	Stimulus	concentration stock (mg/mL)	concentration needed (mg/mL)	concentration needed (mg/mL) * 2	Dilution factor	total wells	total volume needed (mL; 100µl/well; incl 10% spare)	Volume stimulus (µL)	Volume DC medium (µL)
Medium only	-	-	-	-		32	3,52	**0**	**3520**
Positive stimulus	PMA	1	0,0005	0,001	1000	32	3,52	**3,52**	**3515,78**
	Ionomycin	10	0,001	0,002	5000	32	3,52	**0,70**	
S protein peptide mix 1	158 peptide	15,8	0,0315	0,062	254,84	64,00	7,04	**27,63**	**6984,57**
S protein peptide mix 2	157 peptide	15,7			253,23	64,00	7,04	**27,80**	
RBD peptides	48 peptides	**1,2**	0,0048	0,0096	125	64	7,04	**56,32**	**6983,68**
the S peptide stocks has a concentration of 100µg/mL per peptide; RBD has 25µg/mL									

Mastermix for blocking reagents

Blocking reagent	Volume needed/well [µL]	Volume needed/mL [µL]	Total wells	Total volume needed (ml; 10 µL/well; incl 10% spare)	Volume blocking (µL)	Volume DC medium (µL)
GolgiStop	0,13	13	192	2,112	**27,46**	**2042,30**
GolgiPlug	0,2	20	192	2,112	**42,24**	

working concentration Stop (1:1500), Plug (1:1000)

10 µL per well

Stimulation protocol:

1. Prepare a 96-well tissue culture (F-well)
2. Add 100 µL of stimulus (or medium) to the according well; “pool” on plates are FACS controls – 100 µL medium is sufficient
3. Add 500,000 cells in DC medium per well (100 µL)
4. Incubate plate for 1 h @ 37°C in 5% CO_2
5. Add 10 µL of blocking reagents
6. Swing plates (5× 8-moves)
7. Incubate for 5 h @37°C in 5% CO_2
8. Proceed with staining protocol or put plates in 4°C o/n

Mastermix for L/D reagents

	Live-dead reagent	Volume needed/well [µL]	Volume needed/mL [µL]	Total wells	Total volume needed (ml; 50 µL/well; incl 5% spare)	Volume L/D (µL)	Volume PBS (µL)
eFluor780	L/D	0,05	1	200	11	**11,00**	**10989,00**
	working concentration Stop is 1:1000						
	50 µL per well						

Antibody mix control 1 mastermix

	Markers (extracellular)	µL per reaction (50 µL)	Total wells	Volume Ab (µL)	Volume FACS buffer	
BV510	CD4	0,25	4	**1,05**	**207,9**	includes 5% spare volume
BV421	CD8	0,25	4	**1,05**		

Antibody mix 1 mastermix

	Markers (extracellular)	µL per reaction (50 µL)	Total wells	Volume Ab (µL)	Volume FACS buffer (µL)	
BV421	CD8a	0,25	192	**50,40**	**9928,8**	includes 5% spare volume
BV510	CD4	0,25	192	**50,40**		
BV786	CD25	0,25	192	**50,40**		

Staining protocol:

Note: work with a 4°C cooled centrifuge

9. Mix cells by pipetting 3× up and down and transfer total volume to 'v' bottom plate
10. Centrifuge at 350 ×g, 5 min
11. Wash cells once with 150-200 µL cold PBS
12. Centrifuge at 350 ×g, 5 min. Discard supernatant
    a. Vortex cells carefully; snap against the wells to support pellet dissolving
13. Stain with L/D reagent 50 µL to each well in PBS at 4°C for 15 min
14. Add 100 µL PBS
15. Centrifuge at 350 ×g, 5 min. Discard supernatant; vortex/snap
16. Add antibody master mix 1/antibody mix 1 controls/FACS buffer to each well 50 µL
17. Incubate 30 min at 4°C
18. Add 100 µL FACS buffer
19. Centrifuge at 350 ×g, 5 min. Discard supernatant; vortex/snap
20. Add 2% Histofix
21. Incubate following protocol a, b, or c:
    a. keep it in 4°C for overnight (up to ~16 h) if not proceeding for intracellular staining immediately
    b. incubate for at least 1 h at 4°C and
        i. proceed for intracellular staining or
    c. incubate for at least 1 h at 4°C

i. add 100 µL PBS
ii. centrifuge at 400 ×g, 5 min. Discard supernatant; vortex/snap
iii. add 100 µL PBS and keep it in 4°C until further usage (within ~36 h)
iv. proceed with intracellular staining protocol

Fc Block mastermix

Fc Block	µL per reaction (25 µL)	Total wells	Volume Ab (µL)	Volume perm buffer (µL)	
CD16/CD32	0,5	200	**105**	**4935**	includes 5% spare volume

Antibody mix control 2 mastermix

	Markers (extracellular)	µL per reaction (50 µL)	Total wells	Volume Ab (µL)	Volume perm buffer (µL)	
PE	CD3	0,25	4	**1,05**	**208,95**	includes 5% spare volume

Antibody mix 2 mastermix

	Markers (intracellular)	µL per reaction (25 µL)	Total wells	Volume Ab (µL)	Volume perm buffer (µL)	
BV711	IL-4	0,25	192	**50,40**	**4717,44**	includes 5% spare volume
PE	CD3	0,25	192	**50,4**		
Alexa 488	TNF-α	0,5	192	**100,80**		
Pe-Cy7	IFN-γ	0,1	192	**20,16**		
APC	IL-2	0,5	192	**100,80**		

Intracellular staining protocol:

1. Centrifuge at 400 ×g, 5 min, 4°C. Discard supernatant, vortex/snap
2. Suspend cells with perm buffer, 150 µL each well
3. Centrifuge at 400 ×g, 5 min, 4°C. Discard supernatant, vortex/snap
4. Add Fc block 25 µL amount, to each well, incubate for 10 min
5. Add 25 µL of the antibody master mix 2/antibody mix 2 controls/FACS buffer to the cells, mix carefully and incubate for another 45 min, 2-8°C
6. Add 100 µL 1× Perm buffer
7. Centrifuge at 400 ×g, 5 min, 4°C. Discard supernatant, vortex/snap
8. Add 200 µL 1× Perm buffer

9. Centrifuge at 400 ×g, 5 min, 4°C. Discard supernatant, vortex/snap
10. Discard the supernatant carefully
11. Resuspend the cells carefully in 100 µL FACS buffer or FACS flow
12. Mix cells by pipetting 3× up and down and transfer total volume to ‘U’ bottom plate for FACS Celesta/HTS acquire
13. Store plate at 4°C until time point of measurement/flow cytometry
    a. Note that the plates should be acquired the same day as the intracellular staining may decrease in signal.

Appendix 6: Statistical Analysis

ELISA

Descriptive statistics, ELISA screening analysis, day 7, S1

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,362	0,544	1,35
Maximum	0,00100	1,22	1,59	2,05
Range	0,00100	0,858	1,05	0,704
Mean	0,000125	0,872	1,14	1,74
SD	0,000354	0,321	0,373	0,245
SEM	0,000125	0,113	0,132	0,0867

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ELISA screening analysis, day 14, S1

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,724	1,09	1,71
Maximum	0,0100	1,53	1,80	2,11
Range	0,0100	0,809	0,713	0,407
Mean	0,00325	1,18	1,45	1,88
SD	0,00388	0,299	0,218	0,143
SEM	0,00137	0,106	0,0772	0,0504

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ELISA screening analysis, day 21, S1

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,815	1,36	1,97
Maximum	0,0100	1,67	2,04	2,33
Range	0,0100	0,858	0,672	0,357
Mean	0,00138	1,24	1,67	2,12
SD	0,00350	0,327	0,219	0,122
SEM	0,00124	0,116	0,0775	0,0430

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 7, S1

ANOVA summary	
F	55,55
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,8562

Please note that commas are used as decimal separators.

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,8714	-1,213 to -0,5301	Yes	****	<0,0001
Buffer vs. 1 µg	-1,135	-1,477 to -0,7940	Yes	****	<0,0001
Buffer vs. 5 µg	-1,745	-2,086 to -1,403	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 14, S1

ANOVA summary	
F	131,1
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9335

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-1,179	-1,425 to -0,9326	Yes	****	<0,0001
Buffer vs. 1 µg	-1,449	-1,695 to -1,202	Yes	****	<0,0001
Buffer vs. 5 µg	-1,875	-2,121 to -1,629	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 21, S1

ANOVA summary	
F	156,3
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9436

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-1,240	-1,496 to -0,9841	Yes	****	<0,0001
Buffer vs. 1 µg	-1,672	-1,928 to -1,417	Yes	****	<0,0001
Buffer vs. 5 µg	-2,115	-2,370 to -1,859	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, ELISA screening analysis, day 7, RBD

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,253	0,869	1,51
Maximum	0,00800	1,57	1,84	2,17
Range	0,00800	1,32	0,971	0,661
Mean	0,00288	0,930	1,38	1,85
SD	0,00270	0,473	0,330	0,221
SEM	0,000953	0,167	0,117	0,0783

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ELISA screening analysis, day 14, RBD

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,920	1,18	1,84
Maximum	0,0130	1,64	1,96	2,39
Range	0,0130	0,720	0,785	0,550
Mean	0,00388	1,28	1,57	2,07
SD	0,00476	0,293	0,265	0,195
SEM	0,00168	0,104	0,0936	0,0690

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ELISA screening analysis, day 21, RBD

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00100	0,747	1,56	2,04
Maximum	0,00400	1,65	1,98	2,40
Range	0,00300	0,907	0,424	0,362
Mean	0,00250	1,20	1,82	2,21
SD	0,00107	0,392	0,150	0,140
SEM	0,000378	0,138	0,0529	0,0493

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 7, RBD

ANOVA summary	
F	52,18
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,8483

Please note that commas are used as decimal separators.

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,9270	-1,311 to -0,5432	Yes	****	<0,0001
Buffer vs. 1 µg	-1,381	-1,765 to -0,9975	Yes	****	<0,0001
Buffer vs. 5 µg	-1,852	-2,236 to -1,468	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 14, RBD

ANOVA summary	
F	128,0
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9320

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-1,274	-1,547 to -1,000	Yes	****	<0,0001
Buffer vs. 1 µg	-1,571	-1,844 to -1,297	Yes	****	<0,0001
Buffer vs. 5 µg	-2,065	-2,338 to -1,791	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA screening analysis, day 21, RBD

ANOVA summary	
F	152,6
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9424

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-1,198	-1,472 to -0,9232	Yes	****	<0,0001
Buffer vs. 1 µg	-1,813	-2,087 to -1,539	Yes	****	<0,0001
Buffer vs. 5 µg	-2,211	-2,485 to -1,937	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, ELISA, IgG concentrations, day 28, S1

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	35,8	117	312
Maximum	0,00	117	289	536
Range	0,00	81,5	172	224
Mean	0,00	73,0	206	393
SD	0,00	29,3	59,3	81,7
SEM	0,00	10,4	21,0	28,9

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ELISA, IgG concentrations, day 28, RBD

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	36,7	161	345
Maximum	0,00	129	298	583
Range	0,00	92,8	137	238
Mean	0,00	83,1	242	449
SD	0,00	34,9	48,5	80,9
SEM	0,00	12,3	17,2	28,6

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA, IgG concentrations, day 28, S1

ANOVA summary	
F	86,02
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9021

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-72,97	-138,3 to -7,684	Yes	*	0,0259
Buffer vs. 1 µg	-205,9	-271,2 to -140,6	Yes	****	<0,0001
Buffer vs. 5 µg	-392,7	-458,0 to -327,4	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ELISA, IgG concentrations, day 28, RBD

ANOVA summary	
F	123,4
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9297

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-83,10	-145,5 to -20,66	Yes	**	0,0072
Buffer vs. 1 µg	-241,7	-304,2 to -179,3	Yes	****	<0,0001
Buffer vs. 5 µg	-448,6	-511,0 to -386,1	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Tukey's multiple comparisons post-test, ELISA, reciprocal serum endpoint titer, S1

ANOVA summary	
F	13,76
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,6276

Tukey's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg (day 14)	-66525	-470716 to 337666	No	ns	0,9987
Buffer vs. 1 µg (day 14)	-84750	-488941 to 319441	No	ns	0,9949
Buffer vs. 5 µg (day 14)	-291300	-695491 to 112891	No	ns	0,3063
Buffer vs. 0.2 µg (day 28)	-163725	-567916 to 240466	No	ns	0,8727
Buffer vs. 1 µg (day 28)	-491775	-895966 to -87584	Yes	**	0,0082
Buffer vs. 5 µg (day 28)	-983850	-1388041 to -579659	Yes	****	<0,0001
0.2 µg (day 14) vs. 1 µg (day 14)	-18225	-422416 to 385966	No	ns	>0,9999
0.2 µg (day 14) vs. 5 µg (day 14)	-224775	-628966 to 179416	No	ns	0,6131
0.2 µg (day 14) vs. 0.2 µg (day 28)	-97200	-501391 to 306991	No	ns	0,9893
0.2 µg (day 14) vs. 1 µg (day 28)	-425250	-829441 to -21059	Yes	*	0,0332
0.2 µg (day 14) vs. 5 µg (day 28)	-917325	-1321516 to -513134	Yes	****	<0,0001
1 µg (day 14) vs. 5 µg (day 14)	-206550	-610741 to 197641	No	ns	0,7009
1 µg (day 14) vs. 0.2 µg (day 28)	-78975	-483166 to 325216	No	ns	0,9965
1 µg (day 14) vs. 1 µg (day 28)	-407025	-811216 to -2834	Yes	*	0,0474
1 µg (day 14) vs. 5 µg (day 28)	-899100	-1303291 to -494909	Yes	****	<0,0001

Tukey's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
5 µg (day 14) vs. 0.2 µg (day 28)	127575	-276616 to 531766	No	ns	0,9580
5 µg (day 14) vs. 1 µg (day 28)	-200475	-604666 to 203716	No	ns	0,7289
5 µg (day 14) vs. 5 µg (day 28)	-692550	-1096741 to -288359	Yes	****	<0,0001
0.2 µg (day 28) vs. 1 µg (day 28)	-328050	-732241 to 76141	No	ns	0,1837
0.2 µg (day 28) vs. 5 µg (day 28)	-820125	-1224316 to -415934	Yes	****	<0,0001
1 µg (day 28) vs. 5 µg (day 28)	-492075	-896266 to -87884	Yes	**	0,0081

One-way ANOVA with Tukey's multiple comparisons post-test, ELISA, reciprocal serum endpoint titer, RBD

ANOVA summary	
F	17,63
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,6834

Tukey's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg (day 14)	-66525	-250933 to 117883	No	ns	0,9224
Buffer vs. 1 µg (day 14)	-78675	-263083 to 105733	No	ns	0,8433
Buffer vs. 5 µg (day 14)	-327750	-512158 to -143342	Yes	****	<0,0001
Buffer vs. 0.2 µg (day 28)	-60450	-244858 to 123958	No	ns	0,9498
Buffer vs. 1 µg (day 28)	-218400	-402808 to -33992	Yes	*	0,0109
Buffer vs. 5 µg (day 28)	-491775	-676183 to -307367	Yes	****	<0,0001
0.2 µg (day 14) vs. 1 µg (day 14)	-12150	-196558 to 172258	No	ns	>0,9999
0.2 µg (day 14) vs. 5 µg (day 14)	-261225	-445633 to -76817	Yes	**	0,0012
0.2 µg (day 14) vs. 0.2 µg (day 28)	6075	-178333 to 190483	No	ns	>0,9999
0.2 µg (day 14) vs. 1 µg (day 28)	-151875	-336283 to 32533	No	ns	0,1707
0.2 µg (day 14) vs. 5 µg (day 28)	-425250	-609658 to -240842	Yes	****	<0,0001
1 µg (day 14) vs. 5 µg (day 14)	-249075	-433483 to -64667	Yes	**	0,0024

Tukey's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
1 µg (day 14) vs. 0.2 µg (day 28)	18225	-166183 to 202633	No	ns	>0,9999
1 µg (day 14) vs. 1 µg (day 28)	-139725	-324133 to 44683	No	ns	0,2513
1 µg (day 14) vs. 5 µg (day 28)	-413100	-597508 to -228692	Yes	****	<0,0001
5 µg (day 14) vs. 0.2 µg (day 28)	267300	82892 to 451708	Yes	***	0,0009
5 µg (day 14) vs. 1 µg (day 28)	109350	-75058 to 293758	No	ns	0,5396
5 µg (day 14) vs. 5 µg (day 28)	-164025	-348433 to 20383	No	ns	0,1112
0.2 µg (day 28) vs. 1 µg (day 28)	-157950	-342358 to 26458	No	ns	0,1385
0.2 µg (day 28) vs. 5 µg (day 28)	-431325	-615733 to -246917	Yes	****	<0,0001
1 µg (day 28) vs. 5 µg (day 28)	-273375	-457783 to -88967	Yes	***	0,0007

Descriptive statistics, IgG subtype-specific ELISA, day 28, IgG1

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,498	1,04	1,71
Maximum	0,00300	1,57	1,70	2,68
Range	0,00300	1,07	0,651	0,966
Mean	0,000375	1,00	1,40	2,20
SD	0,00106	0,399	0,229	0,319
SEM	0,000375	0,141	0,0811	0,113

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, IgG subtype-specific ELISA, day 28, IgG2a

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,00	0,206	0,811	2,21
Maximum	0,00100	1,48	2,37	2,68
Range	0,00100	1,27	1,56	0,462
Mean	0,000125	0,640	1,82	2,48
SD	0,000354	0,399	0,515	0,167
SEM	0,000125	0,141	0,182	0,0589

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, IgG subtype-specific ELISA, day 28, IgG1

ANOVA summary	
F	85,19
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9013

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-1,004	-1,351 to -0,6567	Yes	****	<0,0001
Buffer vs. 1 µg	-1,396	-1,743 to -1,049	Yes	****	<0,0001
Buffer vs. 5 µg	-2,197	-2,544 to -1,849	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, IgG subtype-specific ELISA, day 28, IgG2a

ANOVA summary	
F	88,60
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9047

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,6398	-1,057 to -0,2221	Yes	**	0,0020
Buffer vs. 1 µg	-1,821	-2,238 to -1,403	Yes	****	<0,0001
Buffer vs. 5 µg	-2,475	-2,893 to -2,057	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, ELISA, IgG2a/IgG1 ratio, day 28

	0.2 µg	1 µg	5 µg
Number of values	8	8	8
Minimum	0,272	0,777	0,827
Maximum	1,27	1,81	1,56
Range	0,998	1,03	0,737
Mean	0,635	1,29	1,15
SD	0,307	0,311	0,227
SEM	0,109	0,110	0,0804

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Tukey's multiple comparisons post-test ELISA, IgG2a/IgG1 ratio, day 28

ANOVA summary	
F	11,92
P value	0,0003
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0,5317

Tukey's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
0.2 µg vs. 1 µg	-0,6595	-1,018 to -0,3009	Yes	***	0,0004
0.2 µg vs. 5 µg	-0,5186	-0,8771 to -0,1600	Yes	**	0,0041
1 µg vs. 5 µg	0,1409	-0,2177 to 0,4995	No	ns	0,5907

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Pseudovirus-based neutralization test

Descriptive statistics, pVNT, day 14

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	6,00	6,00	12,0	48,0
Maximum	6,00	96,0	192	192
Range	0,00	90,0	180	144
Mean	6,00	21,8	46,5	90,0
SD	0,00	31,1	59,6	47,6
SEM	0,00	11,0	21,1	16,8

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, pVNT, day 21

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	6,00	6,00	48,0	96,0
Maximum	6,00	96,0	384	384
Range	0,00	90,0	336	288
Mean	6,00	30,8	144	228
SD	0,00	31,3	112	102
SEM	0,00	11,1	39,5	36,0

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, pVNT, day 28

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	6,00	12,0	96,0	192
Maximum	6,00	96,0	384	384
Range	0,00	84,0	288	192
Mean	6,00	33,0	192	312
SD	0,00	27,8	88,9	99,4
SEM	0,00	9,82	31,4	35,1

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, pVNT, day 14

ANOVA summary	
F	6,330
P value	0,0021
P value summary	**
Significant diff. among means (P < 0.05)?	Yes
R square	0,4041

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-15,75	-66,89 to 35,39	No	ns	0,7862
Buffer vs. 1 µg	-40,50	-91,64 to 10,64	No	ns	0,1440
Buffer vs. 5 µg	-84,00	-135,1 to -32,86	Yes	***	0,0010

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, pVNT, day 21

ANOVA summary	
F	14,28
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,6047

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-24,75	-120,6 to 71,14	No	ns	0,8582
Buffer vs. 1 µg	-138,0	-233,9 to -42,11	Yes	**	0,0036
Buffer vs. 5 µg	-222,0	-317,9 to -126,1	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, pVNT, day 28

ANOVA summary	
F	35,44
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,7916

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-27,00	-111,5 to 57,54	No	ns	0,7684
Buffer vs. 1 µg	-186,0	-270,5 to -101,5	Yes	****	<0,0001
Buffer vs. 5 µg	-306,0	-390,5 to -221,5	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

ELISpot analysis

Descriptive statistics, day 28, fresh splenocytes, S protein

	Buffer control	0.2 µg	1 µg
Number of values	8	8	8
Minimum	6,00	228	858
Maximum	13,7	626	1334
Range	7,67	399	477
Mean	8,54	334	1054
SD	2,72	134	177
SEM	0,961	47,4	62,6

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, day 28, fresh splenocytes, RBD

	Buffer control	0.2 µg	1 µg
Number of values	8	8	8
Minimum	1,67	20,0	33,0
Maximum	8,00	73,0	126
Range	6,33	53,0	93,3
Mean	4,46	36,3	87,3
SD	1,90	19,6	29,2
SEM	0,672	6,93	10,3

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, fresh splenocytes, S protein

ANOVA summary	
F	139,2
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,9299

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-325,0	-477,0 to -173,0	Yes	***	0,0001
Buffer vs. 1 µg	-1045	-1197 to -893,3	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, fresh splenocytes, RBD

ANOVA summary	
F	33,83
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,7631

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-31,83	-55,90 to -7,763	Yes	**	0,0094
Buffer vs. 1 µg	-82,79	-106,9 to -58,72	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, day 28, frozen splenocytes, S protein

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,667	24,7	157	51,7
Maximum	4,67	137	277	232
Range	4,00	112	121	180
Mean	2,13	76,4	206	139
SD	1,49	35,9	52,3	66,7
SEM	0,527	12,7	18,5	23,6

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, day 28, frozen splenocytes, RBD

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,333	1,33	12,3	4,67
Maximum	4,67	20,0	33,0	42,7
Range	4,33	18,7	20,7	38,0
Mean	2,63	9,38	21,8	17,9
SD	1,20	6,72	7,21	12,0
SEM	0,425	2,38	2,55	4,25

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, frozen splenocytes, S protein

ANOVA summary	
F	28,56
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,7537

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-74,25	-131,4 to -17,11	Yes	**	0,0087
Buffer vs. 1 µg	-203,6	-260,8 to -146,5	Yes	****	<0,0001
Buffer vs. 5 µg	-136,5	-193,6 to -79,32	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, frozen splenocytes, RBD

ANOVA summary	
F	9,726
P value	0,0001
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0,5103

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-6,750	-16,43 to 2,933	No	ns	0,2210
Buffer vs. 1 µg	-19,17	-28,85 to -9,484	Yes	***	0,0001
Buffer vs. 5 µg	-15,25	-24,93 to -5,567	Yes	**	0,0015

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, day 28, ELISpot after MACS

	CD4			CD8		
	AH-1	RBD	S	AH-1	RBD	S
Number of values	8	8	8	8	8	8
Minimum	0,00	1,50	66,0	1,00	21,5	173
Maximum	4,50	23,0	155	3,50	62,5	477
Range	4,50	21,5	89,0	2,50	41,0	304
Mean	1,81	13,0	98,8	2,19	43,6	287
SD	1,62	7,75	34,6	0,843	15,6	104
SEM	0,574	2,74	12,2	0,298	5,51	36,9

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, ELISpot after MACS, $CD4^+$

ANOVA summary	
F	53,66
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,8363

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
AH-1 vs. RBD	-11,19	-35,47 to 13,10	No	ns	0,4568
AH-1 vs. S	-97,00	-121,3 to -72,71	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, day 28, ELISpot after MACS, $CD8^+$

ANOVA summary	
F	51,01
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,8293

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
AH-1 vs. RBD	-41,44	-113,6 to 30,70	No	ns	0,3119
AH-1 vs. S	-284,5	-356,6 to -212,4	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Intracellular cytokine staining

Descriptive statistics, ICS, $CD4^+$, IFN-γ

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0500	0,110	0,115	0,0730
Maximum	0,104	0,175	0,220	0,240
Range	0,0540	0,0650	0,105	0,167
Mean	0,0858	0,145	0,159	0,119
SD	0,0172	0,0220	0,0317	0,0550
SEM	0,00606	0,00779	0,0112	0,0194

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ICS, $CD4^+$, IL-4

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0750	0,0695	0,0780	0,0590
Maximum	0,110	0,129	0,120	0,184
Range	0,0350	0,0590	0,0420	0,125
Mean	0,0934	0,107	0,101	0,0968
SD	0,0116	0,0180	0,0163	0,0471
SEM	0,00411	0,00638	0,00577	0,0166

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ICS, $CD4^+$, TNF-α

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0740	0,0785	0,150	0,130
Maximum	0,170	0,155	0,250	0,265
Range	0,0960	0,0765	0,100	0,135
Mean	0,0992	0,113	0,190	0,193
SD	0,0305	0,0243	0,0317	0,0514
SEM	0,0108	0,00858	0,0112	0,0182

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ICS, $CD4^+$, IL-2

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0685	0,110	0,130	0,0995
Maximum	0,130	0,235	0,220	0,660
Range	0,0615	0,125	0,0900	0,561
Mean	0,0968	0,158	0,175	0,223
SD	0,0210	0,0365	0,0331	0,195
SEM	0,00741	0,0129	0,0117	0,0689

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, $CD4^+$, IFN-γ

ANOVA summary	
F	20
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,66

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,059	-0,088 to -0,030	Yes	***	0,0002
Buffer vs. 1 µg	-0,073	-0,10 to -0,044	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, CD4+, IL-4

ANOVA summary	
F	1,6
P value	0,2304
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R square	0,13

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. ns: Not significant.

No post-test for insignificant main test.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, CD4+, TNF-α

ANOVA summary	
F	23
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,69

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,013	-0,048 to 0,021	No	ns	0,5633
Buffer vs. 1 µg	-0,091	-0,13 to -0,056	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, CD4+, IL-2

ANOVA summary	
F	14
P value	0,0001
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R square	0,57

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,061	-0,097 to -0,024	Yes	**	0,0015
Buffer vs. 1 µg	-0,078	-0,11 to -0,042	Yes	***	0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

Descriptive statistics, ICS, CD8+, IFN-γ

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0340	0,190	0,435	0,210
Maximum	0,102	0,700	0,875	0,645
Range	0,0680	0,510	0,440	0,435
Mean	0,0594	0,361	0,704	0,463
SD	0,0223	0,162	0,141	0,134
SEM	0,00789	0,0572	0,0500	0,0472

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ICS, CD8+, TNF-α

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,104	0,270	0,450	0,270
Maximum	0,205	0,750	0,945	0,670
Range	0,102	0,480	0,495	0,400
Mean	0,150	0,410	0,723	0,524
SD	0,0350	0,162	0,154	0,134
SEM	0,0124	0,0571	0,0543	0,0475

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

Descriptive statistics, ICS, CD8+, IL-2

	Buffer control	0.2 µg	1 µg	5 µg
Number of values	8	8	8	8
Minimum	0,0585	0,165	0,170	0,135
Maximum	0,117	0,290	0,400	0,555
Range	0,0580	0,125	0,230	0,420
Mean	0,0840	0,214	0,286	0,268
SD	0,0198	0,0504	0,0794	0,172
SEM	0,00699	0,0178	0,0281	0,0608

Please note that commas are used as decimal separators. SD: Standard deviation. SEM: Standard error of the mean.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, $CD8^+$, IFN-γ

ANOVA summary	
F	54
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,84

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,30	-0,45 to -0,15	Yes	***	0,0002
Buffer vs. 1 µg	-0,64	-0,79 to -0,50	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, $CD8^+$, TNF-α

ANOVA summary	
F	39
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,79

Dunnett's multiple comparisons test	Mean diff.	95,00% CI of diff.	Significant?	Summary	Adjusted P value
Buffer vs. 0.2 µg	-0,26	-0,41 to -0,11	Yes	**	0,0013
Buffer vs. 1 µg	-0,57	-0,73 to -0,42	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

One-way ANOVA with Dunnett's multiple comparisons post-test, ICS, $CD8^+$, IL-2

ANOVA summary	
F	27
P value	<0,0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0,72

Dunnett's multiple comparisons test	**Mean diff.**	**95,00% CI of diff.**	**Significant?**	**Summary**	**Adjusted P value**
Buffer vs. 0.2 µg	-0,13	-0,20 to -0,064	Yes	***	0,0003
Buffer vs. 1 µg	-0,20	-0,27 to -0,14	Yes	****	<0,0001

Please note that commas are used as decimal separators. F: F-statistic. P values ≤ 0.05 indicate statistically significant difference. R square: Coefficient of determination. CI: Confidence interval. n.s.: Not significant.

(b) (6)

Von: (b) (6)
Gesendet: Montag, 23. November 2020 14:17
An: (b) (6)
Cc: (b) (6)
Betreff: Final R-20-0085 version 4.0 report, with signatures
Anlagen: R-20-0085 modRNA V9_Report_V4.0_signatures.pdf

Kennzeichnung: Zur Nachverfolgung
Kennzeichnungsstatus: Gekennzeichnet

Hi (b) (6),

Attached here is the final updated PDF for R-20-0085 version 4. With this email, I'm giving my approval as Author. I'll wet-ink sign this document when I'm next in Mainz.

Best,
(b) (6)

(b) (6)
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(b) (6)

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R&D STUDY REPORT No. R-20-0112

CHARACTERIZING THE IMMUNOPHENOTYPE IN SPLEEN AND LYMPH NODE OF MICE TREATED WITH SARS-COV-2 VACCINE CANDIDATES

Version 01
Date: 13 AUG 2020

Reported by (b) (6)

Test item: BNT162a1, BNT162b1, BNT162b2, BNT162c2
Key words: Covid-19, SARS-CoV-2, Vaccine, BALB/c mice, immunophenotyping

This R&D report consists of 105 pages.

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LIST OF ABBREVIATIONS

Ab	Antibody
CP	Cytoplasmic domain
dLNs	Draining lymph nodes
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FM	Fluorescence minus
FP	Fusion peptide
GC	Germinal center
HR1, HR2	Heptad repeats 1 and 2
i.m.	Intramuscular
IFNγ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IL	Iliac
IN	Inguinal
LD	LiveDead viability dye
LN	Lymph node
LNPs	Lipid nanoparticles
Lot	Lot number
MM	Master mix
modRNA	Nucleoside modified mRNA
NEAA	Non-essential amino acids
PO	Popliteal
RBD	Receptor binding domain
RBM	Receptor binding motif
S	Spike protein
saRNA	Self-amplifying RNA
SP	Signal peptide
T_{FH}	Follicular helper T cells
T_H	T helper cells
TM	Transmembrane domain
TNF	Tumor necrosis factor
uRNA	Non-modified uridine-containing mRNA

RESPONSIBILITIES

Person responsible for the study:	(b) (6)	27 Aug 2020
	(b) (6) BioNTech RNA Pharmaceuticals	Date
Author:	(b) (6)	27 Aug 2020
	(b) (6) BioNTech RNA Pharmaceuticals	Date
Reviewer:	(b) (6)	27 AUG 2020
	BioNTech RNA Pharmaceuticals	Date
QA representative	(b) (6)	27AUG2020
	BioNTech SE	Date

Meaning of the signatures:

Person responsible for the study: I am responsible for the content of the R&D report and confirm that it represents an accurate record of the results. This study was performed according to the SOPs and methods as well as the rules and regulations described in the report.

Author: I am the author of this document.

Reviewer: I reviewed the R&D report and confirm that this document complies with the scientific and technical standards and requirements.

QA representative: I confirm that this document complies with the relevant quality assurance requirements.

1 SUMMARY

BioNTech is developing RNA-based vaccines designed to protect against the novel coronavirus disease that emerged in 2019 (COVID-19). The project involves testing three RNA platforms, which are under development at BioNTech, with the surface or spike (S) protein of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the viral antigen.

In the present study, T- and B-cell responses as well as the ability of $CD8^+$ T cells to kill viral antigen-presenting cells induced by four clinical SARS-CoV-2 vaccine candidates were characterized.

The study was divided into two parts, with the first part characterizing the vaccine candidates BNT162a1 and BNT162b1, and the second part characterizing BNT162b2 and BNT162c2. For each part, eight BALB/c mice per group were vaccinated with 5 µg of RNA encapsulated in lipid nanoparticles (LNPs) or buffer control on day 0 by intramuscular injection. T and B cells were analyzed seven days after vaccination in the blood. Serum for optional determination of SARS-CoV-2 specific IgG responses was stored, spleen and the draining lymph nodes (dLNs) were analyzed after 12 days (BNT162a1, BNT162b1 and BNT162b2), or 27 days (BNT162c2). Splenocytes were used for IFNγ ELISpot assay and xCELLigence cytotoxicity assay, and cell suspensions prepared from dLNs and spleen were analyzed by flow cytometry. Cytokines produced by restimulated dLN and spleen cells were analyzed by ProcartaPlex cytokine multiplex assay.

IFNγ ELISpot revealed a strong S protein specific T-cell responses particularly in BNT162b2, BNT162b1 and BNT162c2 and to a lesser extent in BNT162a1 treated groups. In line, $CD8^+$ and $CD4^+$ T cells in dLNs were significantly increased after BNT162b2 treatment, the former already detectable at day 7 in the blood. A trend for increased T cell numbers was detected in the BNT162b1 and BNT162a1 groups. Particular BNT162b1 and BNT162b2 treatment resulted in T cell activation (CD44, CD38, PD1 and ICOS expression of T cells in blood) and antigen specific secretion of cytokines by splenocytes. In those groups, a predominant T_H1 phenotype was detected with increased numbers of T-bet^+ $CD4^+$ T cells, high secretion of T_H1 type cytokines (IFNγ, IL-2, TNF) and low secretion of T_H2 type cytokines (IL-4, IL-5). In all analyzed compartments BNT162b1, BNT162b2 and BNT162c2 treatment mediated the increase and activation of T_{FH} cells, a cell type known for its crucial support of B cell responses. B cell numbers in dLNs were significantly elevated after BNT162b1 and BNT162b2 treatment with higher numbers of antibody producing plasma B cells, class switched and germinal center B cells essential for affinity maturation of antibodies.

Due to the prominent induction of both T and B cell responses, these results particularly support further clinical evaluation of the SARS-CoV-2 vaccine candidates BNT162b1 and BNT162b2.

(b) (6)	27 Aug 2020
BioNTech RNA Pharmaceuticals	Date

2 GENERAL INFORMATION

2.1 Sponsor and Test Facilities

Sponsor

BioNTech RNA Pharmaceuticals GmbH
An der Goldgrube 12
55131 Mainz
Germany

Test Facility

BioNTech SE
An der Goldgrube 12
55131 Mainz
Germany

2.2 Participating Pers

Responsible person: (as defined in SOP-100-024)	(b) (6) BioNTech RNA Pharmaceuticals GmbH An der Goldgrube 12 55131 Mainz
Author:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH

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Experimenter:	(b) (6) BioNTech RNA Pharmaceuticals GmbH

2.3 Study Dates

Start of experiments: 06 MAY 2020

Completion of experiments: 04 JUN 2020

2.4 Guidelines and Regulations

All experiments are executed in accordance with the existing standard operating procedures and described processes from BioNTech SE. Applicable documents are listed below.

- Animal test application approval number: G18-12-100, Amendment from 24.04.2020 (approved 30.04.2020).
- SOP-010-015 Pipetten und Dispenser
- SOP-010-017 Brutschränke - Biolytics
- SOP-010-028 Vi-Cell XR
- SOP-010-045 Brutschrank HERAcell 150i
- SOP-010-047 Zentrifuge Eppendorf 5810/5810R
- SOP-010-051 Tiefkühlschränke -80°C
- SOP-010-058 Sicherheitswerkbank Klasse II
- SOP-010-086 Zentrifuge Thermo Scientific Heraeus Pico und Fresco 17
- SOP-010-099 CTL ELISPOT Reader
- SOP-010-128 FACSCelesta
- SOP-020-009 Ansetzen von Medien und Zusätzen für die Zellkultur
- SOP-030-038 Standardisierte Kultivierung von Zellen
- SOP-030-041 Auftauen von Zellen
- SOP-030-050 Elektroporation von Zellen
- SOP-030-051 Selektion mit MACS MicroBeads
- SOP-030-054 Extrazelluläre Färbung für Durchflusszytometrie
- SOP-030-071 Abtöten von Mäusen
- SOP-030-072 Fixiergriff und Ohrmarkierung bei Mäusen
- SOP-030-073 Betäubung bei Mäusen
- SOP-030-074 Blutentnahme bei Mäusen
- SOP-030-078 Isolierung muriner Splenozyten
- SOP-030-079 Intramuskuläre Applikation bei Mäusen
- SOP-030-110 IFNγ ELISpot (murin)
- SOP-090-013 Biological safety in laboratories
- SOP-110-022 Entsorgung von Biostoffabfällen

2.5 Changes and Deviations

This R&D study was conducted according to R&D plan P-20-0112. Table 1 summarizes all changes and deviations to the R&D plan.

Table 1: Changes and deviations to R&D study plan

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
mCorVAC#15	xCELLigence cytotoxicity assay	Duplicates for all samples	Duplicates only for some samples	Not enough cells per mouse	Some samples were assayed in singlicates
mCorVAC#15	Flow cytometry	Functional and phenotypic T cell analysis, dLN: 2×10^6 cells/well	Functional and phenotypic T cell analysis, dLN: 1×10^6 cells/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results	None.
mCorVAC#15	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: Use cells in 200 µL for acquisition	Phenotypic T cell analysis, SP: Group 1 mouse 1: 160 µL sample acquired by device without recording. To the remaining sample volume, 160 µL flow buffer were then added and the sample re-acquired and recorded.	Deviation	Total recordable living cell number for Group1 mouse 1 was 133,209 instead of the intended minimum of 1,000,000.
mCorVAC#15, mCorVAC#16	Flow cytometry	Analysis of SP and dLNs on day 12 (day 27)	Analysis of blood 7 days after immunization	Gain further information on the T and B cell immunophenotype early after immunization	Blood phenotyping data available for 7 days after immunization.
mCorVAC#15, mCorVAC#16	ELISpot	Use MultiScreenHTS plates (Millipore)	Precoated ELISpot plates were used (Mabtech)	bring in line with previous mCorVac experiments	None.
mCorVAC#15, mCorVAC#16	Flow cytometry, cytokine multiplex assay	Restimulation with S peptide mixes at 0.1 µg/mL per peptide	Restimulation with S peptide mixes at 0.2 µg/mL per peptide (mCorVAC#15 and mCorVAC#16) and at 0.5 µg/mL per peptide (flow cytometry, mCorVAC#16)	Increase peptide concentration to increase sensitivity and pick up low responses	Direct quantitative comparison of cytokine profiles derived from multiplex assay of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable
mCorVAC#15, mCorVAC#16	Flow cytometry (functional T cell analysis), cytokine	dLN: All samples	dLN: Not all samples included in assay	Not enough cells per mouse	Lower sample numbers for this assay

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
	multiplex analysis				
mCorVAC#15, mCorVAC#16	Flow cytometry	Brefeldin A and GolgiStop in medium were added to a final concentration of 10 µg/mL brefeldin A and a dilution of GolgiStop of 1:500.	GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000.	GolgiPlug contains brefeldin A and replaced brefeldin A solution. Dilution of GolgiStop was lowered as this lower dilution was found to be sufficient for its purpose.	None.
mCorVAC#15, mCorVAC#16	Flow cytometry	Extracellular and intracellular/intra nuclear staining for phenotypic and functional T cell analysis was performed alike and was the same for mCorVAC#15 and mCorVAC#16. B and myeloid cell staining was not described in detail.	Extracellular and intracellular/intranuclear staining for phenotypic and functional T cell analysis varied compared to the study plan and was again altered between mCorVAC#15 and mCorVAC#16 with respect to antibody panels, washing procedures, fixation procedures and centrifugation conditions (see sections 4.5.11.2, 4.5.11.3 and 4.5.11.4 for details). B and myeloid cell staining was performed according to section 4.5.11.5 and 4.5.11.6.	Improve the quality of the results. Detailed stianing protocol for B and myeloid cells.	Direct quantitative comparison of T and B cell flow cytometry data of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable.
mCorVAC#15, mCorVAC#16	Flow cytometry (myeloid cell analysis)	dLN: All samples	dLN: No samples included in assay	Not enough cells per mouse	Assay was not performed for dLN
mCorVAC#15, mCorVAC#16	Cytokine multiplex assay	dLN: 5×10^5/well	dLN: 4×10^5/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, dLN: 2×10^6 cells/well.	Phenotypic T cell analysis, dLN: 1.5×10^6 cells/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results.	None.
mCorVAC#16	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	xCELLigence cytotoxicity assay	Using CT26 cells electroporated	S RNA electroporated CT26 cells were loaded with S peptide mix after electroporation	Improve the quality of the results	None.

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
		with S RNA as targets			
mCorVAC#15	xCELLigence cytotoxicity assay	Duplicates for all samples	Duplicates only for some samples	Not enough cells per mouse	Some samples were assayed in singlicates
mCorVAC#15	Flow cytometry	Functional and phenotypic T cell analysis, dLN: 2 × 10^6 cells/well	Functional and phenotypic T cell analysis, dLN: 1 × 10^6 cells/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: 2 × 10^6/well	Phenotypic T cell analysis, SP: 4 × 10^6/well	Improve the quality of the results	None.
mCorVAC#15	Flow cytometry	B cell analysis, dLN, SP: 2 × 10^6/well	B cell analysis, dLN: 2.5 × 10^5/well. SP: 1 × 10^6/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: Use cells in 200 µL for acquisition	Phenotypic T cell analysis, SP: Group 1 mouse 1: 160 µL sample acquired by device without recording. To the remaining sample volume, 160 µL flow buffer were then added and the sample re-acquired and recorded.	Deviation	Total recordable living cell number for Group1 mouse 1 was 133,209 instead of the intended minimum of 1,000,000.
mCorVAC#15, mCorVAC#16	Flow cytometry	Analysis of SP and dLNs on day 12 (day 27)	Analysis of blood 7 days after immunization	Gain further information on the T and B cell immunophenotype early after immunization	Blood phenotyping data available for 7 days after immunization.
mCorVAC#15, mCorVAC#16	ELISpot	Use MultiScreenHTS plates (Millipore)	Precoated ELISpot plates were used (Mabtech)	bring in line with previous mCorVac experiments	None.
mCorVAC#15, mCorVAC#16	Flow cytometry, cytokine multiplex assay	Restimulation with S peptide mixes at 0.1 µg/mL per peptide	Restimulation with S peptide mixes at 0.2 µg/mL per peptide (mCorVAC#15 and mCorVAC#16) and at 0.5 µg/mL per peptide (flow cytometry, mCorVAC#16)	Increase peptide concentration to increase sensitivity and pick up low responses	Direct quantitative comparison of cytokine profiles derived from multiplex assay of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable
mCorVAC#15, mCorVAC#16	Flow cytometry (functional T cell analysis), cytokine	dLN: All samples	dLN: Not all samples included in assay	Not enough cells per mouse	Lower sample numbers for this assay

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
	multiplex analysis				
mCorVAC#15, mCorVAC#16	Flow cytometry	Brefeldin A and GolgiStop in medium were added to a final concentration of 10 µg/mL brefeldin A and a dilution of GolgiStop of 1:500.	GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000.	GolgiPlug contains brefeldin A and replaced brefeldin A solution. Dilution of GolgiStop was lowered as this lower dilution was found to be sufficient for its purpose.	None.
mCorVAC#15, mCorVAC#16	Flow cytometry	Extracellular and intracellular/intra nuclear staining for phenotypic and functional T cell analysis was performed alike and was the same for mCorVAC#15 and mCorVAC#16. B and myeloid cell staining was not described in detail.	Extracellular and intracellular/intranuclear staining for phenotypic and functional T cell analysis varied compared to the study plan and was again altered between mCorVAC#15 and mCorVAC#16 with respect to antibody panels, washing procedures, fixation procedures and centrifugation conditions (see sections 4.5.11.2, 4.5.11.3 and 4.5.11.4 for details). B and myeloid cell staining was performed according to section 4.5.11.5 and 4.5.11.6.	Improve the quality of the results. Detailed stianing protocol for B and myeloid cells.	Direct quantitative comparison of T and B cell flow cytometry data of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable.
mCorVAC#15, mCorVAC#16	Flow cytometry (myeloid cell analysis)	dLN: All samples	dLN: No samples included in assay	Not enough cells per mouse	Assay was not performed for dLN
mCorVAC#15, mCorVAC#16	Cytokine multiplex assay	dLN: 5×10^5/well	dLN: 4×10^5/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, dLN: 2×10^6 cells/well.	Phenotypic T cell analysis, dLN: 1.5×10^6 cells/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results.	None.
mCorVAC#16	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse.	Lower sample numbers for this assay
mCorVAC#16	xCELLigence cytotoxicity assay	Using CT26 cells electroporated	S RNA electroporated CT26 cells were loaded with S peptide mix after electroporation	Improve the quality of the results	None.

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
		with S RNA as targets			
Experiment	**Assay**	**Plan**	**Change/Deviation**	**Reason for change/deviation**	**Implications**
mCorVAC#15	xCELLigence cytotoxicity assay	Duplicates for all samples	Duplicates only for some samples	Not enough cells per mouse	Some samples were assayed in singlicates
mCorVAC#15	Flow cytometry	Functional and phenotypic T cell analysis, dLN: 2×10^6 cells/well	Functional and phenotypic T cell analysis, dLN: 1×10^6 cells/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: 2×10^6/well	Phenotypic T cell analysis, SP: 4×10^6/well	Improve the quality of the results	None.
mCorVAC#15	Flow cytometry	B cell analysis, dLN, SP: 2×10^6/well	B cell analysis, dLN: 2.5×10^5/well. SP: 1×10^6/well	Not enough cells per mouse	Lower sample numbers for this assay
mCorVAC#15	Flow cytometry	Phenotypic T cell analysis, SP: Use cells in 200 µL for acquisition	Phenotypic T cell analysis, SP: Group 1 mouse 1: 160 µL sample acquired by device without recording. To the remaining sample volume, 160 µL flow buffer were then added and the sample re-acquired and recorded.	Deviation	Total recordable living cell number for Group1 mouse 1 was 133,209 instead of the intended minimum of 1,000,000.
mCorVAC#15, mCorVAC#16	Flow cytometry	Analysis of SP and dLNs on day 12 (day 27)	Analysis of blood 7 days after immunization	Gain further information on the T and B cell immunophenotype early after immunization	Blood phenotyping data available for 7 days after immunization.
mCorVAC#15, mCorVAC#16	ELISpot	Use MultiScreenHTS plates (Millipore)	Precoated ELISpot plates were used (Mabtech)	bring in line with previous mCorVac experiments	None.
mCorVAC#15, mCorVAC#16	Flow cytometry, cytokine multiplex assay	Restimulation with S peptide mixes at 0.1 µg/mL per peptide	Restimulation with S peptide mixes at 0.2 µg/mL per peptide (mCorVAC#15 and mCorVAC#16) and at 0.5 µg/mL per peptide (flow cytometry, mCorVAC#16)	Increase peptide concentration to increase sensitivity and pick up low responses	Direct quantitative comparison of cytokine profiles derived from multiplex assay of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable
mCorVAC#15, mCorVAC#16	Flow cytometry (functional	dLN: All samples	dLN: Not all samples included in assay	Not enough cells per mouse	Lower sample numbers for this assay

Experiment	Assay	Plan	Change/Deviation	Reason for change/deviation	Implications
	T cell analysis), cytokine multiplex analysis				
mCorVAC#15, mCorVAC#16	Flow cytometry	Brefeldin A and GolgiStop in medium were added to a final concentration of 10 µg/mL brefeldin A and a dilution of GolgiStop of 1:500.	GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000.	GolgiPlug contains brefeldin A and replaced brefeldin A solution. Dilution of GolgiStop was lowered as this lower dilution was found to be sufficient for its purpose.	None.
mCorVAC#15, mCorVAC#16	Flow cytometry	Extracellular and intracellular/intranuclear staining for phenotypic and functional T cell analysis was performed alike and was the same for mCorVAC#15 and mCorVAC#16. B and myeloid cell staining was not described in detail.	Extracellular and intracellular/intranuclear staining for phenotypic and functional T cell analysis varied compared to the study plan and was again altered between mCorVAC#15 and mCorVAC#16 with respect to antibody panels, washing procedures, fixation procedures and centrifugation conditions (see sections 4.5.11.2, 4.5.11.3 and 4.5.11.4 for details). B and myeloid cell staining was performed according to section 4.5.11.5 and 4.5.11.6.	Improve the quality of the results. Detailed stianing protocol for B and myeloid cells.	Direct quantitative comparison of T and B cell flow cytometry data of mCorVac#15 (BNT162a1 and BNT162b1) and mCorVac#16 (BNT162b2 and BNT162c2) no longer advisable.
mCorVAC#15, mCorVAC#16	Flow cytometry (myeloid cell analysis)	dLN: All samples	dLN: No samples included in assay	Not enough cells per mouse	Assay was not performed for dLN
mCorVAC#15, mCorVAC#16	Cytokine multiplex assay	dLN: 5×10^5/well	dLN: 4×10^5/well	Not enough cells per mouse	Lower sample numbers for this assay

2.6 Documentation and Archive

Study plans and reports are stored and archived according to SOP-100-003 Archiving of Paper-Based Documents.

Raw data and evaluated data are saved at:

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\00_Pharmacology\mCorVac#15_modRNA_uRNA_V5_dLN_SP

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\00_Pharmacology\mCorVac#16_saRNAV9_modRNAV9_dLN_SP
- Lab book #1934, page 16-80

3 INTRODUCTION

3.1 Background

In December 2019, an outbreak of pneumonia of unknown cause in Wuhan, Hubei province in China, started. The disease spread rapidly and in January 2020, the agent was identified. By July 27th 2020, infection with the novel Coronavirus SARS-CoV-2 was confirmed in approximately 16,100,000 people with more than 640,000 casualties[1]. A vaccine is urgently needed against the elicited coronavirus disease 19 (COVID-19) and BioNTech decided to initiate a rapid vaccine project based on the surface or spike (S) protein of the virus as the viral antigen. The S protein is a trimer and during viral egress, the precursor protein is cleaved into S1 and S2 (Figure 1). While the S1 domain recognizes the host receptor, the S2 domain is essential for membrane fusion of the viral envelope and the endosomal membrane. To initiate membrane fusion, the S2 domain undergoes a conformational change within the central helix domain.

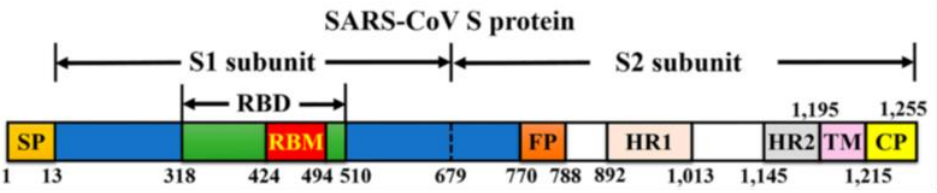

Figure 1: Schematic overview of the S protein structure of the SARS-CoV S protein

The sequence within the S1 subunit consists of the signal peptide (SP) and the receptor binding domain (RBD) with its receptor binding motif (RBM). The S2 subunit contains the fusion peptide (FP) for membrane fusion, heptad repeats (HR1 and HR2), the transmembrane domain (TM) and a cytoplasmic domain (CP). Source: modified from Song et al. 2019.

Based on these features, the S protein is the target of neutralizing antibodies that bind predominantly the receptor-binding domain (RBD) of the S protein.

The development of *in vitro* transcribed RNA as an active platform for the use in infectious disease vaccines is based on the extensive knowledge of the company in RNA technology, which has been gained over the last decade. The core innovation is based on *in vivo* delivery of a pharmacologically optimized, antigen-coding RNA vaccine to induce robust neutralizing antibodies and concomitant T-cell responses to achieve protective immunization with minimal vaccine doses (Vogel et al. 2018, Pardi et al. 2017, Moyo et al. 2019).

At BioNTech, there are three different RNA platforms under development, which are non-modified uridine containing mRNA (uRNA), nucleoside modified mRNA (modRNA) and self-amplifying RNA (saRNA). It is unknown today which RNA vaccine

[1] Coronavirus disease (COVID-2019) situation report 189, World Health Organization; https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

platform performs best in terms of activation and duration of a potent immune response. Initial studies in mice demonstrated the induction of T-cell responses as well as SARS-CoV-2-specific (neutralizing) IgG antibodies with vaccine candidates of all platforms. Four of these candidates are currently tested in clinical trials (Table 2).

The BNT162 vaccine candidate RNA is encapsulated into lipid nanoparticles (LNPs), which protect the RNA from degradation and enable transfection of host cells after intramuscular (i.m.) injection. For all of the BNT162 vaccine candidates, the same LNP formulation is used.

Table 2: Clinical stage SARS-CoV-2 vaccine candidates developed at BioNTech

ID	RNA platform	Antigen
BNT162a1	uRNA	RBD of S1S2 protein (V5)
BNT162b1	modRNA	RBD of S1S2 protein (V5)
BNT162b2	modRNA	S1S2 full-length protein, sequence variant (V9)
BNT162c2	saRNA	S1S2 full-length protein, sequence variant (V9)

This report covers a mouse study characterizing the immunophenotype in the blood, spleen and lymph nodes of mice treated with these four SARS-CoV-2 vaccine candidates.

3.2 Objectives

The objective of this study was to further characterize the four clinical SARS-CoV-2 vaccine candidates to support fast clinical development and approval. In particular, the goal of this study was to:

- Characterize T- and B-cell responses in the spleen, lymph nodes and blood. Analysis included a thorough phenotypic and functional (cytokine secretion on the cellular level) characterization of cells by ELISPot and flow cytometry, and definition of the cytokine profile by multiplex protein quantitation. In particular, the subtype of SARS-CoV-2-specific $CD4^+$ T cells (T_H1, T_H2, T_{FH}) and the abundance of plasma and germinal center (GC) B cells were of interest. Characterize changes in the myeloid cell compartment.
- Determine the ability of $CD8^+$ T cells to kill cells presenting the vaccine-encoded antigen.
- Collect serum of mice to determine (neutralizing) antibody responses (collection was performed, analysis of samples may be performed in the future, if required).

3.3 Study Design

The study was separated into two parts characterizing the vaccine candidates BNT162a1 and BNT162b1 (mCorVac#15, Figure 2) and BNT162b2 and BNT162c2 (mCorVac#16, Figure 3). Each part compared the effects of vaccinated mice to a

control group receiving buffer only. Eight BALB/c mice per group were vaccinated once (day 0) and blood analyzed 7 days later. Serum and tissues were analyzed 12 days later. Since T-cell responses of mice vaccinated with saRNA (BNT162c2) take longer to develop, the analysis time point for serum and tissues was postponed to day 27 after vaccination.

Blood, spleen and draining lymph nodes (dLNs) were harvested from mice. Figure 4 shows an overview of the subsequent analytical methods including sample allocation to the respective assays.

- Serum was obtained from blood and stored frozen for optional determination of SARS-CoV-2 specific IgG responses.
- Splenocytes were tested for recognition of an S protein-specific peptide mix or S RNA-electroporated CT26 cells by secretion of IFNγ (IFNγ ELISpot assay).
- A fraction of splenocytes (N=3 only for the control group, N=8 for treatment groups) was restimulated overnight with an S protein-specific peptide mix and recombinant IL-2, and isolated $CD8^+$ T cells were challenged on the next day for killing of S RNA-electroporated CT26 colon carcinoma cells (xCELLigence cytotoxicity assay).
- Splenocytes and dLN (popliteal, iliac and inguinal, pooled) cells were analyzed for T- ($CD4^+$ T_H1, T_H2, T_{FH}, $CD8^+$ T cells) and B-cell phenotype (GC, plasma, memory B cells), T-cell cytokine secretion after restimulation with an S protein-specific peptide mix, and myeloid cell subsets (flow cytometry).
- dLN and spleen cells were restimulated for 48 h with an S protein-specific peptide mix to analyze T-cell secreted cytokines in the supernatant (ProcartaPlex cytokine multiplex assay).

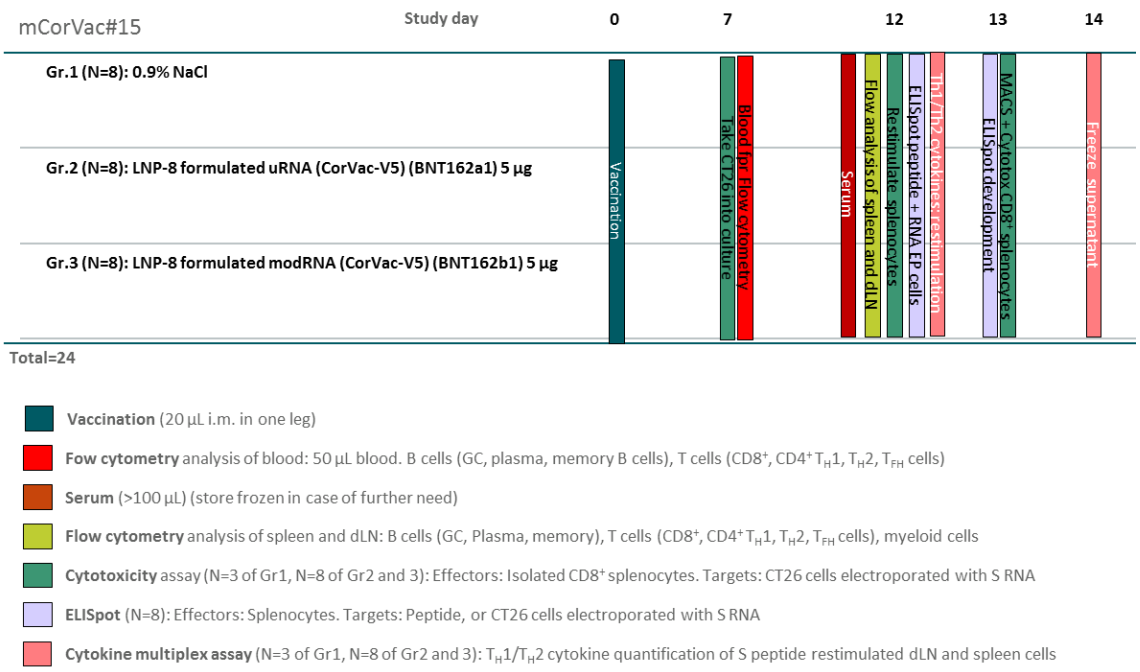

Figure 2: Workflow of part 1 of the study (mCorVac#15)

dLN, draining lymph node. GC, germinal center. Gr, group. N, number of mice per group. T_H, T helper cells. T_{FH}, follicular T helper cells.

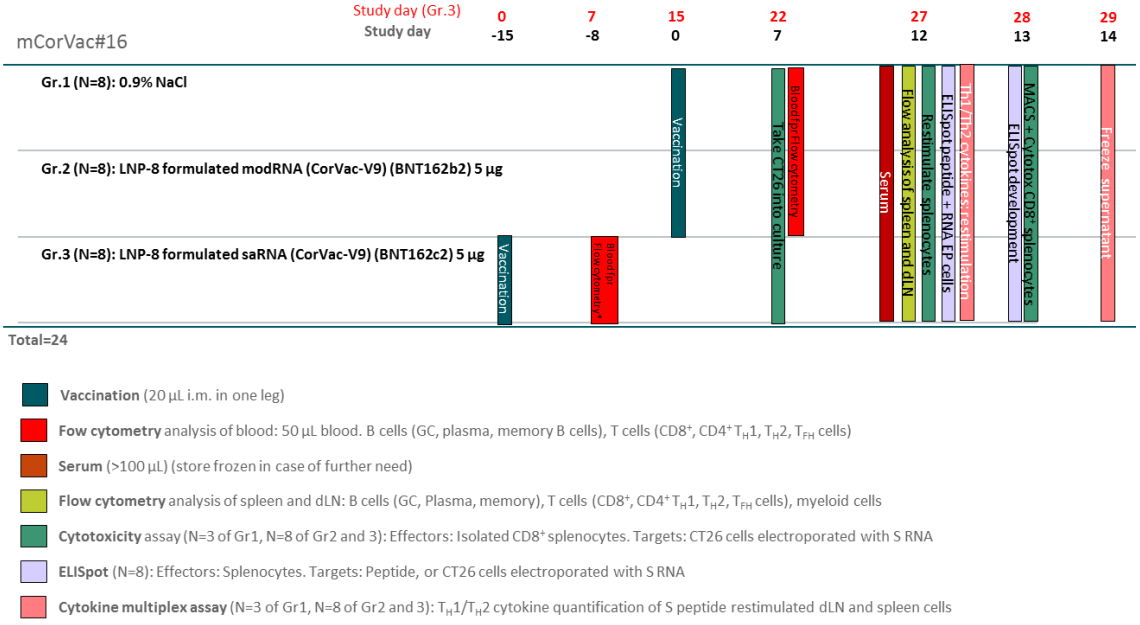

Figure 3: Workflow of part 2 of the study (mCorVac#16)

Study dates for group 3 are depicted in red. dLN, draining lymph node. GC, germinal center. Gr, group. N, number of mice per group. T_H, T helper cells. T_{FH}, follicular T helper cells.

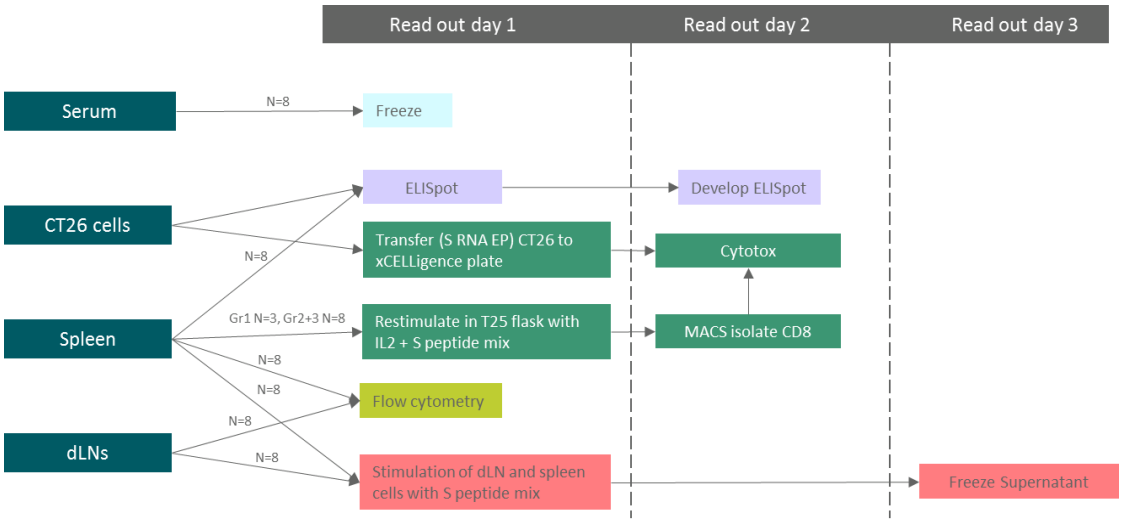

Figure 4: Analysis and assay overview

Schematic depiction of sample allocation to different analysis methods and their timing during analysis days 1 to 3. dLN, draining lymph node. EP, electroporated.

4 MATERIALS AND METHODS

4.1 Test Item

BNT162a1 (ATM): For CoAs see Appendix 9.6

- RNA batch: RNA-SK200305-01
- Polymun batch RBL063.3 LNP with the lot: CoVVAC/090320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162b1 (ATM): For CoAs see Appendix 9.6.

- RNA batch: RNA-RF200304-03
- Polymun batch RBP020.3 LNP with the lot: CoVVAC/100320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162b2 (ATM): For CoAs see Appendix 9.8.

- RNA batch: RNA-RF200321-06
- Polymun batch RBP20.2 LNP with the lot: CoVVAC/270320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.5 mg/mL

BNT162c2 (ATM): For CoAs see Appendix 9.9.

- RNA batch: RNA-RF200310-01
- Polymun batch RBS004.2 LNP with the lot: CoVVAC/170320
- Dilution buffer: 0.9% NaCl
- Concentration: 0.3 mg/mL

Test items are diluted to 0.25 mg/mL with sterile 0.9% NaCl before administration.

4.2 Control Item

- 0.9% NaCl

4.3 Test System

- 48 female BALB/c mice with approximately nine weeks of age at study start

4.4 Materials

For antibodies used in flow cytometry, refer to Section 4.5.11.

Table 3: Materials

Product name	Application/specification	Article no.	Working dilution	Provider
15 mL/50 mL tube	Conical bottom, PP, 30/115 MM, CELLSTAR®	188271/ 227261	N/A	Greiner Bio-One GmbH
2 mL tube	CRYO.S, round bottom	122278	N/A	Greiner Bio-One GmbH
2-Mercaptoethanol	50 mM	31350-010	N/A	Gibco
4mL Sample Cup	Cell counting	NC9756824	N/A	Beckman Coulter GmbH
8-channel manifold	Polypropylene	BR704526-1EA	N/A	Sigma-Aldrich Chemie GmbH
96-well Microplate	Clear round bottom TC-treated microplate, with lid, sterile	3799	N/A	Corning Holding GmbH
ACK lysis buffer	Flow cytometry (blood)	A10492-01	1x	Gibco
Ammonium chloride	NH_4Cl	A0988,5000	N/A	AppliChem GmbH
Brilliant Stain Buffer	Flow cytometry	563794	N/A	BD Bioscience
Brilliant Stain Buffer Plus	Flow cytometry	566385	N/A	BD Biosciences
Capillary pipettes	minicaps®, blood sampling, 4 µL/ 10 µL, not heparinized	9000104/ 9000110	N/A	Hirschmann Laborgeräte GmbH & Co.KG
Cell culture flask 250 ML, 75 cm^2	Cell culture	658175	N/A	Greiner Bio-One GmbH
CD8a (Ly-2) MicroBeads	CD8 T cell purification	130-117-044	N/A	Miltenyi Biotec
Collagenase D	Lymphnode preparation	11088866001	1 mg/ml	Merck KGaA
Combitips advanced®	Biopur®, 50 mL	0030089693	N/A	Eppendorf Vertrieb Deutschland GmbH
Concanavalin A	from Canavalia ensiformis (Jack bean, 5mg),Type IV-S, lyophilized	C0412-5MG	2 µg/mL	Sigma-Aldrich Chemie GmbH
Dimethyl sulfoxide	Cell culture	A3672,0100	N/A	AppliChem GmbH

Product name	Application/specification	Article no.	Working dilution	Provider
DPBS	No calcium, no magnesium	14190-094	1 ×	Thermo Fisher Scientific
Easystrainer 70 µm	For 50 mL tubes	542070	N/A	Greiner Bio-One GmbH
Electroporation cuvette	Electroporation	732-1137	N/A	VWR International GmbH
E-Plate VIEW 96 PET	xCelligence	300600910	N/A	ACEA Biosciences
Eppendorf safe-lock tubes	0.5 mL/ 1.5 mL/ 2.0 mL/ 5.0 mL, Eppendorf Quality™	0030121023/ 0030120086/ 0030120094/ 0030119401	N/A	Eppendorf Vertrieb Deutschland GmbH
Ethylenediaminetetraacetic acid solution	EDTA	03690-100ML	N/A	Sigma-Aldrich Chemie GmbH
Fetal Bovine Serum	Non-USA origin, sterile-filtered	F7524	N/A	Sigma-Aldrich Chemie GmbH
Filtration unit for medium flasks	High Performance, PES, 0.45 µm, 1000 mL	514-0301	N/A	VWR International GmbH
FoxP3/Transcription Factor Staining Buffer Set	Flow cytometry	00-5523-00	N/A	Thermo Fisher Scientific
GolgiStop	Flow cytometry (Restimulation)	554724	1:1,500	BD Biosciences
GolgiPlug	Flow cytometry (Restimulation)	555029	1:1,000	BD Biosciences
Heparin Tubes	Flow cytometry (Blood)	20.1309	N/A	Sarstedt AG & Co.
HEPES	1 M	15630-056	N/A	Gibco
Insulin syringes	BD Micro-Fine™+, 30 G, 0.3 mL	324826	N/A	Becton Dickinson GmbH
Ionomycin, 10 µg/µL	Flow cytometry (Restimulation)	I9657	1 µg/mL	Sigma
Isoflurane	Anesthesia	9714675	N/A	Piramal Critical Care
Isotonic saline	Injection solution	06173569	N/A	Fresenius Kabi Deutschland GmbH
LS columns	CD8 T cell purification	130-042-401	N/A	Miltenyi Biotec

Product name	Application/specification	Article no.	Working dilution	Provider
MEM Non-Essential Amino Acids Solution (100X)	Cell culture	11140-035	1X	Gibco
Mouse IFN-γ ELISpot^{PLUS} kit	Kit for enumeration of cells secreting mouse IFN-γ	3321-4APT-2	N/A	Mabtech
PBS dry substance	No calcium, no magnesium	L182-10	N/A	Merck KGaA
Penicillin-Streptomycin	10,000 U/mL	15140-122	N/A	Gibco
PepMix™ against RBD	ELISpot	N/A (customized)	0.0625mg per peptide/ vial	JPT
Pipette tips	ep Dualfilter T.I.P.S.®, PCR clean und sterile, 0.1–10 µL/2–100 µL/50–1000 µL/50–1250 µL/0.1–5 mL	0030077512/ 0030077547/ 0030077555/ 0030077792/ 0030077750/ 0030078616	N/A	Eppendorf Vertrieb Deutschland GmbH
PMA, 1 µg/µL	Flow cytometry (Restimulation)	P1585	0.5 µg/mL	Sigma
Potassium bicarbonate	$KHCO_3$	A2375,1000	N/A	AppliChem GmbH
ProcartaPlex mouse T_H1/T_H2 cytokine 11-plex kit	Cytokine multiplex assay Lot. No. 232634-004	EPX110-20820-901	N/A	Thermo Fisher Scientific
Proleukin S	Cell culture	N/A	100 U/ml	Clinigen
Reservoir	25 mL, 100 mL	613-1174/613-1171	N/A	VWR International GmbH
RotiHistofix	Flow cytometry	P087.1	2%	Roth
Round bottom 5-mL tubes	Flow cytometry (blood)	10579511	N/A	Thermo Fisher Scientific
RPMI 1640 Medium	GlutaMAX™ Supplement	61870-010	N/A	Gibco
Serological pipettes	5 mL, 10 mL, 25 mL, 50 mL	606180/607180/601180/768180	N/A	Greiner Bio-One GmbH
Serum Tubes	Serum preparation	20.1344	N/A	Sarstedt AG & Co.
Single-use syringe	Injekt® Solo 5 mL	4606051V	N/A	B. Braun Melsungen AG

Product name	Application/specification	Article no.	Working dilution	Provider
Sodium acide (10%)	Flow cytometry (blood)	13553.00100	0.01%	Morphisto
Sodium Pyruvate	100 mM	11360-039	N/A	Gibco
StemPro™ Accutase™ Cell dissociation reagent	Cell culture	A1110501	N/A	Gibco
Sterile filters	0.45 µm	514-4123	N/A	VWR International
Vi-CELL™ XR Quad Pak	For Vi-CELL™ XR Cell Viability Analyzer	383722	N/A	Beckman Coulter GmbH
X-VIVO 15, serum-free	Electroporation	BE02-060Q	N/A	Lonza Group Ltd

Table 4: Peptide pool for restimulation of splenocytes and dLN cells for ELISpot assays, flow cytometry and cytokine multiplex assay

S protein-specific peptides	
Name	**Sequence**
2019-nCoV S.wt With a total of 315 overlapping peptides (15mers overlapping by 11 amino acids) GenBank: QHD43416.1 Batch: 43000LHB-1 and 43000LHB-2	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQ DLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWI FGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEF RVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPI NLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAA AYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTS NFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLY NSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYN YKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQA GSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCG PKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRD PQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRA RSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCT MYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTP PIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAA RDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAM QMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVN QNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTY VTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHG VVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYE PQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVD LGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLG FIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHY T

Table 5: RNAs used for CT26 electroporation

Name	Sequence (open reading frame)
S RNA (Full construct name: JR206_mRNA_pST4_m1ψ_wtsec_SARS-CoV-2-S (Sino CDS) CΔ19)	atgtttgtgttcctggtgctgctgccactggtgtccagccagtgtgtgaacctgaccaccaggacccaacttcctcctgcctaca ccaactccttcaccaggggagtctactaccctgacaaggtgttcaggtcctctgtgctgcacagcacccaggacctgttcctg ccattcttcagcaatgtgacctggttccatgccatccatgtgtctggcaccaatggcaccaagaggtttgacaaccctgtgctgc cattcaatgatggagtctactttgccagcacagagaagagcaacatcatcaggggctggatttttggcaccaccctggacag caagacccagtccctgctgattgtgaacaatgccaccaatgtggtgattaaggtgtgtgagttccagttctgtaatgacccattc ctgggagtctactaccacaagaacaacaagtcctggatggagtctgagttcagggtctactcctctgccaacaactgtaccttt gaatatgtgagccaaccattcctgatggacttggagggcaagcagggcaacttcaagaacctgagggagtttgtgttcaag aacattgatggctacttcaagatttacagcaaacacacaccaatcaacctggtgagggacctgccacagggcttctctgcctt ggaaccactggtggacctgccaattggcatcaacatcaccaggttccagaccctgctggctctgcacaggtcctacctgaca cctggagactcctcctctggctggacagcaggagcagcagcctactatgtgggctacctccaaccaaggaccttcctgctga aatacaatgagaatggcaccatcacagatgctgtggactgtgccctggacccactgtctgagaccaagtgtaccctgaaatc cttcacagtggagaagggcatctaccagaccagcaacttcagggtccaaccaacagagagcattgtgaggtttccaaacat caccaacctgtgtccatttggagaggtgttcaatgccaccaggtttgcctctgtctatgcctggaacaggaagaggattagca actgtgtggctgactactctgtgctctacaactctgcctccttcagcaccttcaagtgttatggagtgagcccaaccaaactgaa tgacctgtgtttcaccaatgtctatgctgactcctttgtgattaggggagatgaggtgagacagattgcccctggacaaacagg caagattgctgactacaactacaaactgcctgatgacttcacaggctgtgtgattgcctggaacagcaacaacctggacagc aaggtgggaggcaactacaactacctctacagactgttcaggaagagcaacctgaaaccatttgagagggacatcagca cagagatttaccaggctggcagcacaccatgtaatggagtggagggcttcaactgttactttccacctccaatcctatggcttcc aaccaaccaatggagtgggctaccaaccatacagggtggtggtgctgtcctttgaactgctccatgcccctgccacagtgtgt ggaccaaagaagagcaccaacctggtgaagaacaagtgtgtgaacttcaacttcaatggactgacaggcacaggagtgc tgacagagagcaacaagaagttcctgccattccaacagtttggcagggacattgctgacaccacagatgctgtgagggac ccacagaccttggagattctggacatcacaccatgttcctttggaggagtgtctgtgattacacctggcaccaacaccagcaa ccaggtggctgtgctctaccaggatgtgaactgtactgaggtgcctgtggctatccatgctgaccaacttacaccaacctgga gggtctacagcacaggcagcaatgtgttccagaccagggctggctgtctgattggagcagagcatgtgaacaactcctatg agtgtgacatcccaattggagcaggcatctgtgcctcctaccagacccagaccaacagcccaaggagggcaaggtctgtg gcaagccagagcatcattgcctacacaatgagtctgggagcagagaactctgtggcttacagcaacaacagcattgccatc ccaaccaacttcaccatctctgtgaccacagagattctgcctgtgagtatgaccaagacctctgtggactgtacaatgtatatct gtggagacagcacagagtgtagcaacctgctgctccaatatggctccttctgtacccaacttaacagggctctgacaggcatt gctgtggaacaggacaagaacacccaggaggtgtttgcccaggtgaagcagatttacaagacacctccaatcaaggactt tggaggcttcaacttcagccagattctgcctgacccaagcaagccaagcaagaggtccttcattgaggacctgctgttcaac aaggtgaccctggctgatgctggcttcatcaagcaatatggagactgtctgggagacattgctgccagggacctgatttgtgc ccagaagttcaatggactgacagtgctgcctccactgctgacagatgagatgattgcccaatacacctctgccctgctggctg gcaccatcacctctggctggacctttggagcaggagcagccctccaaatcccatttgctatgcagatggcttacaggttcaat ggcattggagtgacccagaatgtgctctatgagaaccagaaactgattgccaaccagttcaactctgccattggcaagattc aggactccctgtccagcacagcctctgccctgggcaaactccaagatgtggtgaaccagaatgcccaggctctgaacacc ctggtgaagcaactttccagcaactttggagccatctcctctgtgctgaatgacatcctgagcagactggacaaggtggaggc tgaggtccagattgacagactgattacaggcagactccaatccctccaaacctatgtgacccaacaacttatcagggctgct gagattagggcatctgccaacctggctgccaccaagatgagtgagtgtgtgctgggacaaagcaagagggtggacttctgt ggcaagggctaccacctgatgagttttccacagtctgcccctcatggagtggtgttcctgcatgtgacctatgtgcctgcccag gagaagaacttcaccacagcccctgccatctgccatgatggcaaggctcactttccaagggagggagtgtttgtgagcaatg gcacccactggtttgtgacccagaggaacttctatgaaccacagattatcaccacagacaacaccttttgtgtctggcaactgt gatgtggtgattggcattgtgaacaacacagtctatgacccactccaacctgaactggactccttcaaggaggaactggaca aatacttcaagaaccacaccagccctgatgtggacctgggagacatctctggcatcaatgcctctgtggtgaacatccagaa ggagattgacagactgaatgaggtggctaagaacctgaatgagtccctgattgacctccaagaactgggcaaatatgaac aatacatcaagtggccatggtacatctggctgggcttcattgctggactgattgccattgtgatggtgaccataatgctgtgttgta tgacctcctgttgttcctgtctgaaaggctgttgttcctgtggctcctgttgttgatga
Irrelevant RNA (Batch: RNA-KG200106-06c)	atgggcgccatggcccctagaacattgctcctgctgctggccgctgccctggcccctacacagacaagagctggacctggc ggctctggaggaggcggctccggaggcggaggatccggtggtggcggcagcggcggcgtgatcgtggctgtgctgggcg tgctgggagccatggccatcattggagccgtggtggccttcgtgatgaagcggagaagaaacaccggcggcaagggcgg cgattacgctctggctcctggcagccagtccagcgagatgagcctgagagactgcaaggcctagtaa

Table 6: Software

Product name	Application	Provider
BD FACSDiva software version 9.1 and 8.0.1.1	Flow cytometry	BD Biosciences
Excel	Raw data	Microsoft Corp.
FlowJo software version 10.6	Flow cytometry	FlowJo LLC, BD Biosciences
GraphPad Prism software version 8	Statistical analysis	GraphPad Software Inc.
ImmunoCapture 7.0.7.0	ELISpot assay	Cellular Technology Ltd
ImmunoSpot® analysis software version 57.0.17.0	ELISpot assay	Cellular Technology Ltd
ProcartaPley Analyst software version 1	Cytokine multiplex assay	Thermo Fisher Scientific
RTCA Data analysis software	xCELLigence cytotoxicity assay	ACEA Biosciences
xCELLigence RTCA Software Pro	xCELLigence cytotoxicity assay	ACEA Biosciences

Table 7: Machines

Product name	Application	Provider
BD Symphony A3	Flow cytometry	BD Biosciences
BD Celesta	Flow cytometry	BD Biosciences
Bioplex200 system	Cytokine multiplex assay	Bio-Rad
Centrifuges	Centrifugation	Eppendorf
CTL ELISPOT reader ImmunoSpot® S6 Core Analyzer	ELISpot assay	Cellular Technology Limited
Electroporation system	Electroporation	BTX
Vi-CELL XR	Cell counting	Beckman Coulter GmbH
xCELLigence RTCA MP	xCELLigence cytotoxicity assay	ACEA Biosciences

4.5 Methods

4.5.1 Animal Care

4.5.1.1 General Information

BALB/c mice were delivered at the age of at least six weeks. Delivered mice were used for experiments after approximately one week of acclimatization. All experiments and protocols were approved by the local authorities (local welfare committee), conducted according to the FELASA recommendations and in compliance with the German animal welfare act and Directive 2010/63/EU. Only animals with an unobjectionable health status were selected for testing procedures.

All animals were registered upon arrival in the lab animal colony management system PyRAT (Scionics Computer Innovation GmbH, Dresden, Germany) and tracked until death. Each cage was labelled with a cage card indicating the mouse strain, gender, date of birth and number of animals per cage. At the start of an experiment additional information was added such as the project and license number, the start of the experiment and details on interventions. Where necessary for identification, animals were arbitrarily numbered with earmarks.

4.5.1.2 Housing Condition and Husbandry

Mice were housed at BioNTech SE's animal facility under barrier and SPF conditions (An der Goldgrube 12, 55131 Mainz) in individually ventilated cages (Sealsafe GM500 IVC Green Line, TECNIPLAST, Hohenpeißenberg, Germany; 500 cm²) with a maximum of five animals per cage. The temperature and relative humidity in the cages and animal unit was kept at 20-24°C and 45-55%, respectively, and the air change (AC) rate in the cages at 75 AC/h. The cages with dust-free bedding made of debarked chopped aspen wood (Abedd LAB & VET Service GmbH, Vienna, Austria, product code: LTE E-001) and additional nesting material were changed weekly. Autoclaved ssniff M-Z food (sniff Spezialdiäten GmbH, Soest, Germany; product code: V1124) and autoclaved water (tap water) were provided *ad libitum* and changed at least once weekly. All materials were autoclaved prior to use.

4.5.2 Animal Monitoring

Routine animal monitoring was carried out daily and included inspection for dead animals and control of food and water supplies. Each animal's health was closely assessed at least once weekly and the results documented in health monitoring sheets. The general physical condition was assessed with regard to the following parameters:

- Body weight change
- Macroscopic assessment of activity level/ behavior

- Macroscopic assessment of general discomfort: drop in body temperature determined by touch and by visual inspection of ears and paws. Ears and paws appear pink in a healthy mouse, white in a mouse with discomfort indicated by reduced blood circulation
- Macroscopic assessment of fur condition and appearance of eyes, inspection of body cavities/ fluids
- Macroscopic assessment of irregularities in breathing ability
- Indication of pain
- Macroscopic assessment for signs of automutilation and or fighting

4.5.3 Animal Treatment

4.5.3.1 Treatment Schedule, Route of Administration, and Dose

The test compounds were administered i.m. once at a dose of 5 µg (see Figure 2 and Figure 3). The control group was treated with buffer only.

4.5.3.2 Immunization

For immunization, prior anesthesia by inhalation of 2.5% isoflurane in oxygen, the injection site (hind leg) was shaved. Buffer or dissolved test item was applied i.m. into the *musculus gastrocnemius* in a volume of 20 µL. After immunization and a short recovery phase from anesthesia, the animals were observed for any immediate signs of discomfort following the immunization procedure.

4.5.3.3 Blood Sampling via the Retro-Orbital Venous Plexus or *Vena Facialis*

Blood was sampled via the retro-orbital venous plexus according to SOP-030-074. In short, mice were anesthetized by inhalation of 2.5% isoflurane in oxygen and held tightly. A thin glass capillary (29 G) was inserted gently through the retro-orbital sinus membrane and blood was collected into either Serum tubes (serum preparation) or Heparin tubes (flow cytometry analysis). After careful removal of the glass capillary, the restraining grip was loosened. Alternatively, blood collection was performed via the *vena facialis* according to SOP-030-074. In short, without prior anesthesia, mice were held tightly and using a lancet, the *vena facialis* was punctured in a precise and short movement. Blood was collected into either Serum tubes (serum preparation) or Heparin tubes (flow cytometry analysis), and the restraining grip was loosened. Blood samples were centrifuged at 10,000 x g and ambient temperature for 5 min and serum transferred to a pre-labeled 0.5 mL reagent tube, to be stored at -20°C.

4.5.4 Endpoint of Experiment / Termination Criteria

Animals were euthanized in accordance with §4 of the German animal welfare act and the recommendation of GV-SOLAS by cervical dislocation or by exposure to carbon dioxide. Additionally, termination criteria applied according to the specification within the respective animal test approval as listed below. Body weight losses exceeding 20%, or a high severity level in any of the parameters found in Section 4.5.2 were on their own sufficient reason for immediate euthanasia.

4.5.4.1 Dissection of Animals and Organ Collection

Following euthanasia, mice were disinfected with 70% ethanol and the dissection was performed starting with an abdominal incision. The spleen and dLNs (popliteal (PO), iliac (IL) and inguinal (IN), see Figure 5) were collected, pooled and stored in PBS on ice for subsequent single cell preparations.

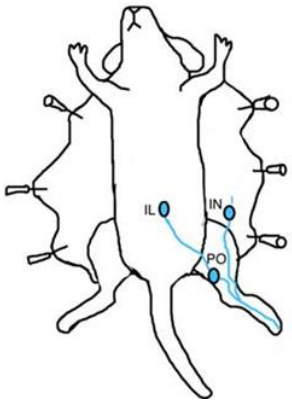

Figure 5: Draining lymph nodes resection for subsequent analysis

Depicted are the predicted draining lymph nodes after i.m. injection into the gastrocnemius muscle used for further analysis. Figure adopted according to **Harrell et al. 2008**. IL, iliac. IN, inguinal. PO, popliteal.

4.5.5 Preparation of Splenocyte Single Cell Suspensions

Single cell suspensions from collected spleens were prepared according to SOP-030-078. To this end, spleens were squeezed through 70 µm cell strainers using the plunger of a syringe to release the splenocytes into a 50 mL tube. Splenocytes were washed with an excess volume of PBS followed by centrifugation at 300 x g for 6 min at ambient temperature and discarding the supernatants. Erythrocytes were lysed with erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 5 min at ambient temperature. The reaction was stopped with an excess volume of PBS. After another washing step, cells were resuspended in DC medium (RPMI medium1640 (1x) + GlutaMAX-I [Life Technologies], 10% FBS, 1% NEAA, 1% sodium-pyruvate, 0.5% penicillin/streptomycin, 50 µM 2-mercaptoethanol), passed through a 70 µm cell strainer again, counted according to SOP-010-028, and stored at 4°C until further use.

4.5.6 Preparation of Lymph Node Single Cell Suspensions

The popliteal, iliac and inguinal dLNs (Figure 5) were stored together in a plastic tube containing 450 µL PBS at ambient temperature in the dark until single cell preparation. 50 µL collagenase D (10 mg/mL) were added to yield a final concentration of 1 mg/mL, the dLNs were thoroughly cut into pieces using forceps or scissors, and incubated for 10 min at 37°C. Cells were passed through a 70 µm cell strainer placed on a 50 mL plastic tube and minced using the plunger of a 5 mL syringe. The cell strainer is subsequently rinsed using 5 mL of PBS and the cell solution counted according to SOP-010-028.

4.5.7 RNA Electroporation

CT26 colon carcinoma cells (ATCC) were washed once with 10 mL of serum-free X-Vivo 15 medium, centrifuged (300 ×g, 6 min, ambient temperature), taken up in 1–2 mL of X-Vivo 15 medium, counted (SOP-010-028), and diluted to a concentration of 25 × 10^6 cells/mL. S Protein encoding modRNA or irrelevant modRNA (10 µg in 40 µL of X-Vivo 15 medium each) was carefully placed at the bottom of a 4 mm electroporation cuvette, topped up with 200 µL of cells (corresponding to 5 × 10^6 cells) and shortly mixed by pipetting up and down. Electroporation was then performed with a BTX™ ECM™ 830 Square Wave Electroporator applying one 300 V pulse for 15 ms. Immediately after electroporation, cells were transferred to a 15 mL tube containing 1–2 mL of DC medium, counted, and diluted to 4 × 10^5 cells/mL for the cytotoxicity assay, and 5 × 10^5 cells/mL for the IFNγ ELISpot assay (Section 4.5.8).

4.5.8 ELISpot Assay

IFNγ ELISpot assay was performed according to SOP-030-110 (with minor modifications as described below) using the mouse IFN-γ ELISpot^{PLUS} kit. Briefly, 96-well ELISpot plates were washed with PBS and blocked with serum-containing medium (DC medium) for at least 30 min at 37°C. After blocking, 100 µl of the splenocyte solution (5 x 10^5 cells) as well as 100 µl electroporated CT26 cells (5 x 10^4 cells) or 100 µl S peptide mix (final concentration per well: 0.1 µg/ml) were added yielding a final volume per well of 200 µL. No peptide or irrelevant RNA transfected cells were used as controls. Plates were incubated overnight in a 37°C humidified incubator with 5% CO_2. After approximately 18 h cells were discarded and a second biotinylated anti-mouse IFN-γ antibody incubated for 2 h at ambient temperature. The plate was then developed by addition of Streptavidin-ALP for 1 h at ambient temperature in the dark followed by addition of BCIP®/NBT substrate for 5–7 min at ambient temperature in the dark. Spots were counted on a CTL ELISPOT reader ImmunoSpot® S6 Core Analyzer according to SOP-010-099.

4.5.9 xCELLigence Cytotoxicity Assay

Preparation of targets:

Of DC medium, 50 µL per well were added to a 96-well PET E-plate to perform a blank measurement at an xCELLigence RTCA MP, Real Time Cell Analyzer. Tumor cells (50 µL of a 4 × 10^5 cells/mL suspension, corresponding to 2 × 10^4 cells) electroporated with S RNA or irrelevant RNA (10 µg each) were subsequently added to the E-plate. After allowing the cell suspension to settle down for 30 min at ambient temperature, the E-plate was transferred to the xCELLigence device and measurement was continued.

Peptide loading of targets:

In mCorVac#16, 100 µl S peptide mix (final concentration per well: 0.1 µg/ml) was added to S RNA electroporated tumor cells one hour prior T cell addition. After one hour of incubation, the medium was carefully aspirated and the wells were washed with PBS twice. Before adding the effector cells, 100 µl of DC medium was dispensed per well.

Addition of effectors:

On the same day, splenocytes were transferred to a T25 cell culture flask at a density of 1.5–2 × 10^6 cells/cm^2. S peptide mixes and recombinant IL-2 (Proleukin) were added to yield a final concentration of 0.1 µg/mL and 100 U/mL, respectively, and the cell suspension was kept at 37°C, 5% CO_2 overnight. On the day after, restimulated splenocytes were transferred to a 15 mL plastic tube, the T25 flask was rinsed with 5 mL of MACS buffer and added to the same tube. Subsequently, $CD8^+$ cells were isolated from restimulated splenocytes using CD8a (Ly-2) MACS® MicroBeads according to the manufacturer's instructions. Labeled cells were eluted from MACS LS columns, centrifuged (5 min at 460 ×g), taken up in 1–2 mL of warm (approximately 37°C) DC medium, counted (SOP-010-028) and diluted with DC medium to a concentration of 6 × 10^6 cells/mL. $CD8^+$ cells (100 µL), DC medium or Staurosporin (4 µM final concentration) were added in duplicate to the targets in the E-plate and the xCELLigence measurement was continued for at least three days. RTCA Data analysis software or xCELLigence RTCA Software Pro (both ACEA Biosciences) were used for data analysis.

4.5.10 Cytokine Multiplex Protein Quantification

Cytokine concentrations were determined in supernatants derived from *ex vivo* restimulated splenocytes and dLN cells. 5 × 10^5 splenocytes or dLN cells in 100 µL medium/well were transferred to a 96-well U-bottom plate, and 100 µL medium supplemented with S peptide mixes to a final concentration of 0.2 µg/mL/peptide/well, or cell culture medium only (negative control) were added and mixed. For each group, three samples were treated with 100 µL PMA and ionomycin to a final concentration of

0.5 µg/mL and 1 µg/mL/well, respectively (positive controls). Cells were incubated for 48 h at 37°C, 5% CO_2. Supernatants were harvested and stored at -20°C for the cytokine multiplex assay.

Cytokine concentrations in supernatants of restimulated splenocytes and dLN cells were determined from thawed cell culture supernatants using a bead-based, 11-plex Th1/Th2 mouse ProcartaPlex immunoassay according to the manufacturer's instructions. Analytes included in the assay were IFNγ, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-6, TNF, GM-CSF, and IL-18.

Fluorescence was measured with the Bioplex200 system and analyzed with ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific).

4.5.11 Flow Cytometry

All flow cytometric data were acquired on a BD Symphony A3 or BD Celesta (B cell analysis) flow cytometer using BD FACSDiva software version 9.1 or 8.0.1.1, respectively, and analyzed with FlowJo 10.6 (FlowJo LLC, BD Biosciences).

4.5.11.1 Restimulation of T cells for functional T cell analysis in the spleen and dLN

For functional analysis, splenocytes and dLN cells were *ex vivo* restimulated. 4×10^6 splenocytes and 1×10^6 (mCorVAC#15) or 2×10^6 (mCorVAC#16) dLN cells in 100 µL DC medium/well were transferred to a 96-well U-bottom plate. To each well, 50 µL medium were added, supplemented with either S peptide mixes to a final concentration of 0.2 µg/mL/peptide/well (mCorVAC#15) or 0.5 µg/mL/peptide/well (mCorVAC#16), or medium only (negative controls), and mixed. To one sample per group, 50 µL PMA and ionomycin to a final concentration of 0.5 µg/mL and 1 µg/mL/well, respectively, were added (positive controls). Three additional wells of any group were added as unstained controls.

Cells were quickly spun down (30 s, 460 × g) and incubated for 1 h at 37°C, 5% CO_2. To each well, 50 µL GolgiStop and GolgiPlug in medium were added to a final dilution of GolgiStop of 1:1,500 and GolgiPlug of 1:1,000, mixed, and cells were further incubated for 4 h at 37°C, 5% CO_2.

4.5.11.2 Functional T cell analysis in the spleen and dLN

For mouse functional T cell analysis, restimulated cells (see 4.5.11.1) were centrifuged (5 min, 300 × g) and supernatants discarded. Flow cytometry master mixes (MM) for functional T cell analysis are depicted in Table 8 and Table 9.

Cells were stained with fixable viability dye and extracellularly with antibodies against CD3, CD4, CD8α, CD44, PD-1, CD40L, CD62L and CXCR5 (mCorVAC#15, MM1a), or CD4, CD8α, CD44, CD45, PD-1, CD40L, CD62L and CXCR5 mCorVAC#16, MM1b)

in Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (PBS supplemented with 2% FCS, 2 mM EDTA, 0.01% sodium azide) in a total volume of 50 µL for 30 min at 2-8 °C. After washing cells once with 200 µL flow buffer (5 min, 300 × g), cells were stained with streptavidin Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (MM2). After washing cells once with 200 µL flow buffer, cells were fixed with 200 µL 2% RotiHistofix for 15 min at room temperature (mCorVAC#15) and resuspended in 200 µL Perm/Wash buffer (FoxP3/Transcription Factor Staining Buffer Set) overnight at 2-8 °C (mCorVAC#15), or overnight at 2-8 °C (mCorVAC#16). After washing cells once with 200 µL Perm/Wash buffer (5 min, 500 × g) (mCorVAC#16), permeabilized cells were intracellulary treated with 25 µL Fc block (diluted 1:50) for 10 min at 2-8 °C before IL-4, TNF, Bcl-6, IFNγ, T-bet and IL-2 antibodies (mCorVAC#15, MM3a) or IL-4, TNF, IFNγ, T-bet, IL-2 and CD3 (mCorVAC#16, MM3b) in Perm/Wash buffer in a total volume of 25 µL were added, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 200 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4 and CD62L (mCorVAC#15), or CD3, CD8a, CD4, CD45 and CD62L (mCorVAC#16) only.

Table 8: Flow cytometry antibody master mixes for functional T cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD	563565	9204644	31.05.2022	100	0,5
BUV563	CD44	IM7	BD	741227	0119427	30.04.2021	2,500	0,1
BV421	CXCR5	L138D7	BioLegend	145512	B281252	L138D7	50	1
BV480	CD4	RM4-5	BD	565634	9016508	31.05.2020	250	0,2
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	30-F11	BD	553079	6197750	31.08.2021	200	0,25
Biotin	CD40L	MR1	BD	553657	8186567	12.04.2024	100	0,5
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM1b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02

BUV737	CD45	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
FITC	CD8	30-F11	BD Biosciences	553079	6197750	31.08.2021	200	0,25
Biotin	CD40L	MR1	BD Biosciences	553657	8186567	12.04.2024	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM2	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE	Streptavidin	N/A	BioLegend	405203	B170498	N/A	200	0,25

MM3a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BV711	IL-4	11B11	BD	564005	9276915	21.03.2021	100	0,5
BB700	TNF	MP6-XT22	BD	566510	0021825	31.03.2021	5,000	0,01
PE	Bcl-6	K112-91	BD	561522	9165931	30.06.2022	50	1
PE-Cy7	IFNγ	XMG1.2	eBioscience	25-731182	E07672-1632	09.2014	1,000	0,05
AF647	T-bet	4B10	biolegend	644804	B248741	N/A	5,000	0,01
APC-R700	IL-2	JES6-5H4	BD	565186	9303906	31.03.2021	5,000	0,01

MM3b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	500	0,10
BV711	IL-4		BD Biosciences	564005	9276915	21.03.2021	100	0,5

BB700	TNF	MP6-XT22	BD Biosciences	566510	0021825	31.03.2021	5,000	0,01
PE-Cy7	IFNγ	XMG1.2	eBioscience	25-731182	E07672-1632	09.2014	1,000	0,05
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01
APC-R700	IL-2	JES6-5H4	BD Biosciences	565186	9303906	31.03.2021	5,000	0,01

4.5.11.3 Phenotypic T cell analysis in the spleen and dLN

For mouse phenotypic T cell analysis in the spleen and dLNs, 4×10^6 splenocytes and 1×10^6 (mCorVAC#15) or 1.5×10^6 (mCorVAC#16) dLN cells/well were transferred to a 96-well U bottom plate, centrifuged (3 min, 300 × g, 2–8°C) and supernatants discarded. Flow cytometry MM for phenotypic T cell analysis are depicted in Table 9.

Cells were stained with fixable viability dye and extracellularly with antibodies against CD3, CD4, CD8α, CD25, CD44, PD-1, CD62L, ICOS, CD19 and CXCR5 (mCorVAC#15, MM1a), or CD4, CD8α, CD25, CD44, CD45, PD-1, CD62L, ICOS, CD19 and CXCR5 (mCorVAC#16, MM1b) in Brilliant Stain Buffer Plus diluted 1:5 in flow buffer (PBS supplemented with 2% FCS, 2 mM EDTA, 0.01% sodium azide) in a total volume of 50 µL for 30 min at 2-8 °C. After washing cells twice with 200 µL flow buffer (5 min, 300 × g), cells were resuspended in 200 µL 2% RotiHistofix, immediately centrifuged (5 min, 300 × g) and fixed again with 200 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) overnight at 2-8 °C (mCorVAC#15), or for 20 min at 2-8 °C and incubated in 200 µL Perm/Wash buffer overnight at 2-8°C (mCorVAC#16). After washing cells once with 200 µL Perm/Wash buffer (5 min, 500 × g) (mCorVAC#15), permeabilized cells were intracellulary treated with 25 µL Fc block (diluted 1:50) for 10 min at room temperature before T-bet, GATA3, FoxP3 and Bcl-6 antibodies (mCorVAC#15, MM2a) or T-bet, GATA3, FoxP3 and CD3 (mCorVAC#16, MM2b) in Perm/Wash buffer in a total volume of 25 µL, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 200 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4, CD62L and CD19 only.

Table 9: Flow cytometry antibody master mixes for phenotypic T cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	565992	9204644	31.05.2022	100	0,50

BUV563	CD44	IM7	BD Biosciences	741227	119427	30.04.2021	2,500	0,02
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25*	PC61	BD Biosciences	740714	119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	53-6.7	BD Biosciences	553031	9143776	31.08.2021	200	0,25
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50
ef780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM1b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02
BUV737	CD45	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	CXCR5	L138D7	BioLegend	145512	B281252	N/A	100	0,50
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B272550	N/A	200	0,25
FITC	CD8	30-F11	BD Biosciences	553031	9143776	31.08.2021	200	0,25
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,000	0,05

MM2a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE	Bcl-6		BD Biosciences	561522	9165931	30.06.2022	100	0,5
PECF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.20.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	2142972	N/A	25	2
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	100	0,5

MM2b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	50	0,50
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.20.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

4.5.11.4 Phenotypic T cell analysis in the blood

For mouse phenotypic T cell analysis in peripheral blood, 50 µL freshly drawn blood were transferred to round bottom 5-mL tubes, washed once with 500 µL PBS (Gibco) (300 × g, 8 min) and the cell pellet was resuspended in 2 mL ACK lysing buffer (Gibco) and incubated for 3 min at room temperature. Flow cytometry master mixes (MM) for phenotypic T cell analysis are depicted in Table 10.

Cells were washed twice with 1 mL flow buffer (300 × g, 8 min) and stained with fixable viability dye and anti-CXCR5 (rat IgG2a) antibody in the presence of Fc block diluted 1:100) in flow buffer in a total volume of 50 µL for 20 min at room temperature (MM1). After washing cells twice with 1 mL flow buffer (8 min, 300 × g), cells were stained with anti-rat IgG2a biotin in flow buffer in a total volume of 50 µL for 20 min at 2-8 °C (MM2). After washing cells twice with 1 mL flow buffer (8 min, 300 × g), cells were stained extracellularly with antibodies against CD3, CD4, CD8α, CD25, CD38, CD44, PD-1, CD62L, ICOS, CD127 (4-1BB), CD19 and streptavidin (mCorVAC#15, MM3a), or CD4, CD8α, CD25, CD38, CD44, PD-1, CD62L, ICOS, CD127 (4-1BB), CD19 and treptavidin (no CD3, mCorVAC#16, MM3b) in Brilliant Stain Buffer Plu diluted 1 5 in flow buffer in a total volume of 50 µL for 20 min at 2-8 °C. After washing cells once with 1 mL flow buffer (5 min, 300 × g), cells were fixed in 200 µL 2% RotiHistofix for 15 min at room temperature (mCorVAC#15), or centrifuged immediately after mixing (5 min,

300 × g) and fixed again with 200 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) for 20 min at 2-8 °C (mCorVAC#16). After centrifugation (5 min, 500 × g), cells were resuspended in 200 µL Perm/Wash buffer (FoxP3/Transcription Factor Staining Buffer Set) and incubated over night at 2-8 °C. Permeabilized cells were centrifuged (5 min, 500 × g) and intracellulary treated with 25 µL Fc block (diluted 1:50) in Perm/Wash buffer for 10 min at 2-8 °C before T-bet and GATA3 antibodies (mCorVAC#15, MM4a) or CD3, FoxP3, T-bet and GATA3 antibodies (mCorVAC#16, MM4b) in Perm/Wash buffer in a total volume of 25 µL were added, and cells incubated for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 1 mL Perm/Wash buffer (5 min, 500 × g), cells were resuspended in 150 µL flow buffer. Fluorescence minus 10 (FM10) controls were stained for viability and with antibodies against CD3, CD8a, CD4, CD62L, CD19 and streptavidin only.

Table 10: Flow cytometry antibody master mixes for phenotypicT cell analysis in the blood (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
Purified	CXCR5	2G8	BD Biosciences	551961	9143926	28.02.2027	100	0,50
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1000	0,05
N/A	Fc block	2.4G2	BD	553142	0028326	31.05.2027	100	0,50

MM2								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
Biotin	IgG2a	RG7/1.30	BD Biosciences	553894	9288614	31.05.2024	100	0,50

MM3a	**mCorVAC#15**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD	563565	9204644	31.05.2022	100	0,50
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2,500	0,02
BUV737	CD8a	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	Streptavidin	N/A	BD Biosciences	563259	9197684	31.12.2021	200	0,25

BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
AF488	CD38	90	BioLegend	102714	B298187	N/A	100	0,50
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
PE	4-1BB	17B5	eBioscience	12-1371-82	E01500-1632	N/A	100	0,50
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5000	0,01
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50

MM3b	**mCorVAC#16**							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV563	CD44	IM7	BD Biosciences	741227	0119427	30.04.2021	2500	0,02
BUV737	CD8a	53-6.7	BD Biosciences	564297	9030634	N/A	200	0,25
BV421	Streptavidin	N/A	BD Biosciences	563259	9197684	31.12.2021	200	0,25
BV480	CD4	RM4-5	BD Biosciences	565634	9016508	31.05.2020	250	0,20
BV605	PD-1	29F.1A12	BioLegend	135219	B281806	N/A	50	1,00
BV711	CD25	PC61	BD Biosciences	740714	0119426	30.04.2021	500	0,10
BV785	CD62L	MEL-14	BioLegend	104440	B258213	N/A	200	0,25
AF488	CD38	90	BioLegend	102714	B298187	N/A	100	0,50
PerCPeF710	ICOS	7E.17G9	Invitrogen	46-9942-82	2029789	30.04.2021	50	1,00
PE	4-1BB	17B5	eBioscience	12-1371-82	E01500-1632	N/A	100	0,50
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5000	0,01
AF700	CD19	6D5	BioLegend	115528	B261756	N/A	100	0,50

MM4a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.02.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

MM4b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD3	145-2C11	BD Biosciences	563565	9204644	31.05.2022	50	0,50
PE-CF594	FoxP3	MF23	BD Biosciences	562466	9276149	28.02.2021	200	0,25
PE-Cy7	GATA3	TWAJ	Invitrogen	25-9966-42	B2142972	N/A	25	2,00
AF647	T-bet	4B10	BioLegend	644804	B248741	N/A	5,000	0,01

4.5.11.5 B cell analysis in the spleen and dLN

For mouse B cell analysis in the spleen and dLNs, 1 × 10^6 splenocytes and 2.5 × 10^5 dLN cells/well were transferred to a 96-well V bottom plate, centrifuged (5 min, 300 × g, 2–8 °C) and supernatants discarded. Flow cytometry MM for B cell analysis are depicted in Table 11.

Cells were treated with Fc block (diluted 1:50) in 50 µL flow buffer for 15 min at 2-8 °C and cells were stained with fixable viability dye and extracellularly with antibodies against CD19, CD45R/B220, IgD, CD138, IgM, CD38, CD95/FAS, IgG1, IgG2a, GR-1, F4/80, CD4 and CD8a (mCorVAC#15, MM1a) in Brilliant Stain Buffer in a total volume of 50 µL for 20 min at 2-8 °C (staining volume: 100 µL); or cells were directly treated with fixable viability dye and extracellularly with antibodies against CD19, CD45R/B220, IgD, CD138, IgM, CD38, CD95/FAS, GR-1, F4/80, CD4 and CD8a (mCorVAC#16, MM1b), in Brilliant Stain Buffer in a total volume of 100 µL for 20 min at 2-8 °C (staining volume: 100 µL). In addition, cells were treated with Fc block (diluted 1:50) in 50 µL flow buffer for 15 min at 2-8 °C and stained with fixable viability dye and extracellularly with antibodies against with PD-L2, CD45R/B220, CD19, CD73, IgM, CD80, GR-1, F4/80, CD4 and CD8a in Brilliant Stain Buffer in a total volume of 50 µL (mCorVAC#15, MM3) (staining volume: 100 µL); or cells were directly treated with fixable viability dye and extracellularly with MM3 (mCorVAC#16) in Brilliant Stain Buffer in a total volume of 100 µL for 20 min at 2-8 °C (staining volume: 100 µL). After washing cells twice with 200 µL flow buffer (5 min, 400 × g, 2–8 °C), cells were fixed

with 200 µL 2% RotiHistofix and incubated over night at 2-8 °C. After washing cells once with 200 µL flow buffer (5 min, 400 × g), cells were resuspended in 100 µL flow buffer (mCorVAC#15), or stained intracellularly with antibodies against IgG1 and IgG2a (MM2) in Perm/Wash buffer in a total volume of 50 µL for 30 min at 2-8 °C (staining volume: 50 µL), before being resuspended in 100 µL flow buffer (mCorVAC#16). Fluorescence minus Fas/CD138 (FM Fas/CD138) controls were stained for MM1a and MM1b excluding CD95/FAS and CD138; fluorescence minus IgG2a (FM IgG2a) controls were stained for MM1a excluding IgG2a; fluorescence minus IgG1 (FM IgG1) controls were stained for MM1a excluding IgG1; fluorescence minus 34 (FM 34) controls were stained for MM1b excluding CD138 and CD95/FAS; fluorescence minus CD73 and CD80 (FM 73/80) controls were stained for MM3 excluding CD73 and CD80; fluorescence minus PD-L2 (FM PD-L2) controls were stained for MM3 excluding PD-L2; and fluorescence minus 35 (FM 35) controls were stained for MM3 excluding PD-L2, CD73 and CD80.

Table 11: Flow cytometry antibody master mixes for B cell analysis in the spleen and dLN (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1a	mCorVAC#15							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
FITC	FAS/CD95	Jo2	BD Biosciences	561979	8296755	30.11.2023	100	1
PE	CD38	90	Thermo Fisher	12-0381-82	2150667	25.04.2021	400	0,25
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD45R/B220	RA3-6B2	BioLegend	103226	B243962	N/A	1,500	0,07
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06
BV421	IgD	11-26c.2a	BioLegend	405725	B280598	N/A	2,500	0,04
BV510	IgG1	A85-1	BD	746811	0115095	30.04.2021	200	0,5
BV605	CD138	281-2	BioLegend	142531	B299222	N/A	200	0,5
BV711	IgG2a	R19-15	BD	744533	0115092	30.04.2021	200	0.5

BV785	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0.1

MM1b	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
FITC	FAS/CD95	Jo2	BD Biosciences	561979	8296755	30.11.2023	100	1
PE	CD38	90	Thermo Fisher	12-0381-82	2150667	25.04.2021	400	0,25
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD45R/B220	RA3-6B2	BioLegend	103226	B243962	N/A	1,500	0,07
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06
BV421	IgD	11-26c.2a	BioLegend	405725	B280598	N/A	2,500	0,04
BV605	CD138	281-2	BioLegend	142531	B299222	N/A	200	0,5
BV785	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0.1

MM2	mCorVAC#16							
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
BV510	IgG1	A85-1	BD	746811	0115095	30.04.2021	400	0,125
BV711	IgG2a	R19-15	BD	744533	0115092	30.04.2021	400	0,125

MM3								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume /test [µL]**
BV421	PD-L2	TY25	BD Biosciences	564245	9204505	30.11.2021	600	0.2

BV605	CD45R/B220	RA3-6B2	BioLegend	103244	B305934	N/A	800	0,12
BV786	CD19	1D3	BD Biosciences	563333	0023948	30.06.2021	1,000	0,1
PE	CD73	TY/11.8	BioLegend	127206	B267137	N/A	600	0,2
PerCPCy5.5	Gr1	RB6-8C5	BioLegend	108428	B278340	N/A	800	0,12
PerCPCy5.5	F4/80	BM8	BioLegend	123128	B276793	N/A	800	0,12
PerCPCy5.5	CD4	RM4-5	BioLegend	100540	B261856	N/A	800	0,12
PerCPCy5.5	CD8	53-6.7	BD Biosciences	551162	9098816	31.05.2023	800	0,12
PE-Cy7	IgM	R6-60.2	BD Biosciences	552867	9269114	18.07.2021	200	0,5
AF647	CD80	16-10A1	BioLegend	104718	B278896	N/A	400	0,25
eF780	LD	N/A	eBioscience	65-0865-14	2178170	N/A	1,600	0,06

4.5.11.6 Myeloid cell analysis in the spleen

For mouse myeloid cell analysis in the spleen, 2×10^6 splenocytes/well were transferred to a 96-well U bottom plate, centrifuged (3 min, 460 × g) and supernatants discarded. Flow cytometry MM for myeloid cell analysis is depicted in Table 12.

Cells were stained with Fc block and fixable viability dye in PBS in a total volume of 100 µL (MM1) for 15 min at 2-8 °C. After washing cells once with 200 µL PBS (3 min, 460 × g), cells were stained extracellularly with antibodies against CD8, CD45, BST2, CD86, XCR1, MHC class II, CD11b, PD-L1, CD103, F4/80, CD11c and GR-1 in Brilliant Stain Buffer in a total volume of 50 µL (MM2) for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells once with 200 µL PBS (3 min, 460 × g), cells were fixed with 100 µL Fix/Perm buffer (FoxP3/Transcription Factor Staining Buffer Set) for 30 min at 2-8 °C. After washing cells twice with 200 µL Perm/Wash buffer (3 min, 460 × g), cells were resuspended in 200 µL Perm/Wash buffer and incubated overnight at 2-8 °C. Permeabilized cells were centrifuged (3 min, 460 × g) and intracellularly treated with CD206 antibody in Perm/Wash buffer in a total volume of 50 µL (MM3) for 30 min at 2-8 °C (staining volume: 50 µL). After washing cells twice with 200 µL Perm/Wash buffer (3 min, 460 × g), cells were resuspended in 200 µL flow buffer.

Table 12: Flow cytometry antibody master mixes for myeloid cell analysis in the spleen (mCorVAC#15 and mCorVAC#16).

Cat, catalog number. LD, LiveDead viability dye. Lot, lot number. MM, master mix.

MM1								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BV605-like	LD	N/A	ThermoFisher	L34959	1921586	N/A	800	0,06

N/A	Fc block	2.4G2	BD	553142	0028326	31.05.2027	100	0,50

MM2								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
BUV395	CD45	30-F11	BD Biosciences	564279	9016570	N/A	100	0,50
BUV737	CD8	53-6.7	BD Biosciences	564297	9030634	N/A	100	0,50
eF450	BST2	eBio927	invitrogen	48-3172-82	2055199	N/A	100	0,50
BV510	CD86	GL-1	BioLegend	105039	B264604	N/A	100	0,50
BV650	XCR1	ZET	BioLegend	148220	B265588	N/A	100	0,50
BV786	MHC II	M5/114.15.2	BD Biosciences	742894	9333783	30.11.2020	500	0,10
FITC	CD11b	M1/70	BD Biosciences	553310	8295813	31.08.2024	200	0,25
PerCP-Cy5.5	PD-L1	10F.9G2	BioLegend	124333	B286738	N/A	100	0,50
PE	CD103	Invitrogen	12-1031-83	2054351	26.12.2021	N/A	400	0,13
PE-Dazzle594	F4/80	BM8	BioLegend	123145	B268244	N/A	100	0,50
APC	CD11c	N418	Miltenyi	130-119-802/130-102-493	5200308676/25200308676	24.3.2021/13.12.2015	100	0,50
APC-Cy7	GR-1	RB-8C5	BioLegend	108423	B209677	N/A	800	0,06

MM3								
Fluorochrome	**Marker**	**Clone**	**Company**	**Cat**	**Lot**	**Expiry**	**Dilution**	**Volume/test [µL]**
PE-Cy7	CD206	C068C2	BioLegend	141719	B260552	N/A	400	0,13

4.5.12 Statistical Analysis

GraphPad Prism 8 Software (La Jolla, USA) was used for statistical analysis and figure generation. The following tests were used for data analysis:

Table 13: Statistical analyses

Data set	Comparison	Statistical test
Flow cytometry, immune cell subsets	Test groups vs. control group	One-way ANOVA and Dunnett's posttest

ELISpot assay	Test groups vs. control group	Repeated measurement one-way ANOVA and Sidak's posttest
Th1/Th2 cytokines	Test groups vs. control group	Two-way ANOVA and Sidak's posttest

5 RESULTS

5.1 ELISpot assay

BALB/c mice were euthanized on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Isolated splenocytes were restimulated with S-specific overlapping peptide mixes (S peptide) or CT26 cells electroporated with RNA encoding the full-length S protein (S RNA). Recognition of S RNA transfected cells served as an additional proof for successful processing of S-specific epitopes. Cells cultivated without the presence of a peptide (No peptide) or control RNA electroporated CT26 cells (Control RNA) served as control. Statistical significance was assessed by repeated measurement one-way ANOVA and Sidak's multiple comparison post-test. Raw data can be found in Table 19.

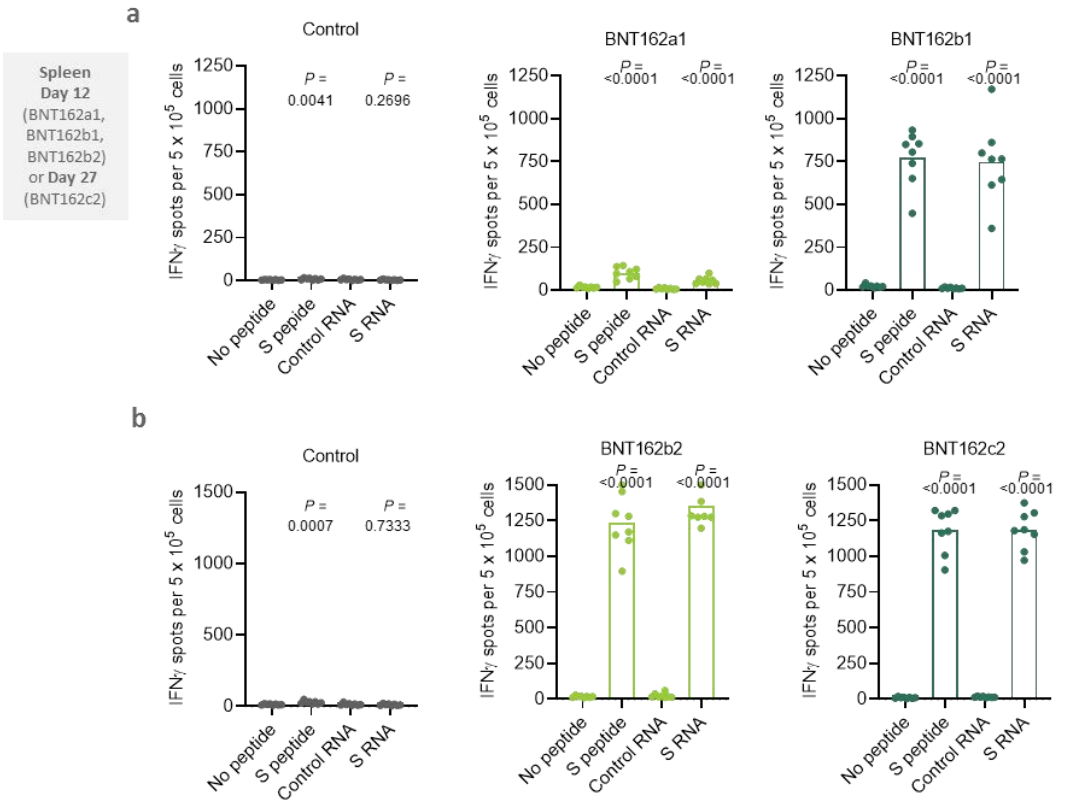

Figure 6: ELISpot analysis using splenocytes from animals treated with BNT162a1, BNT162b1, BNT162b2 or BNT162c2

ELISpot assay of splenocytes from BNT162a1 or BNT162b1 (**a**) or BNT162b2 or BNT162c2 (**b**) vaccinated mice (n=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes were restimulated with S-specific overlapping peptide mixes and IFNγ secretion was measured to assess T-cell responses. Mean spot counts per mouse are shown by dots; group mean values are indicated by bars. One sample in the BNT162b2 group in response to S peptide and S RNA restimulation yielded results that were too numerous to count; these values were set to 1,500.

Saturating amounts of IFNγ spots were detected in groups receiving BNT162b1, BNT162b2 or BNT162c2 after restimulation with either S peptide or S RNA. Mean spot counts were as high as 750 for BNT162b1 and exceeded 1,000 for BNT162b2 and BNT162c2. Low but significant spot counts were detected for BNT162a1, reaching a mean of 100 after S peptide restimulation and 36 after S RNA restimulation.

5.2 Flow Cytometry

Flow cytometry was applied to further characterize T- and B-cell numbers, activation status, functional profile and subtypes after vaccination in the blood, spleen and dLNs. dLNs were analyzed for functionality but are not further described in this report. Myeloid cell subsets in the spleen were analyzed but are not further described in this report. dLNs were not assayed for myeloid cell subsets due to insufficient cell numbers (for further details see Section 2.5). Statistical significance comparing the vaccinated groups to the respective control group was determined by one-way ANOVA and Dunnett's multiple comparison post-test. Raw data for analyzed immune cell subsets including tissues and subsets not described here can be found in Attachment I. Gating strategies can be found in Attachment II.

Phenotypic T- and B-cell analysis in the blood

Blood was analyzed 7 days after vaccination. The $CD8^+$ T cell percentage among $CD3^+$ T cells in the blood was significantly increased around 45% to a mean of 34% for BNT162b2 treated mice with a corresponding decrease in $CD4^+$ T cells (Figure 7a,b). No change in the percentage of $CD8^+$ or $CD4^+$ T cells among $CD3^+$ T cells was observed in any other group. A significant increase of T_{FH} cells among $CD4^+$ T cells was observed in the BNT162b1, BNT162b2 and BNT162c2 groups (Figure 7c). Highest T_{FH} levels with a mean of 1.34% were found for BNT162c2 followed by BNT162b2 (0.53%) and BNT162b1 (0.48%).

Among lymphocytes, B cell levels were significantly reduced in all groups, suggesting a redistribution from the blood into secondary lymphoid organs (Figure 7d).

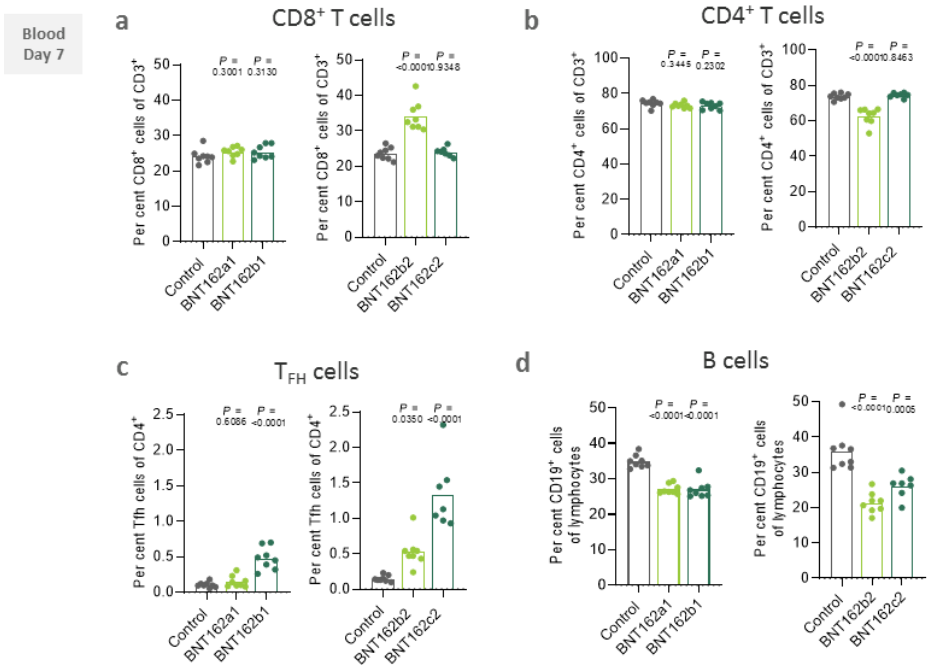

Figure 7: Analysis of lymphocyte frequencies in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of blood 7 days post BNT162a1, BNT162b1, BNT162b2 or BNT162c2 treatment (N=8 per group). Buffer treated mice served as control. For BNT162c2, the control group of mCorVAC#15 served as control (sample processing and acquisition on the same day). Cell fractions per mouse are shown by dots; group mean values are indicated by bars.

The fraction of activated T cells was particularly elevated when mice were treated with BNT162b1 or BNT162b2. In these groups, $CD8^+$ T cells significantly upregulated CD44, CD38, PD-1 as well as ICOS (Figure 8a). ICOS expression was also elevated among $CD4^+$ T cells (Figure 8b). The fraction of $ICOS^+$ T_{FH} cells was increased in all vaccinated groups but most significantly for BNT162b1, BNT162b2 and BNT162c2 (Figure 8c).

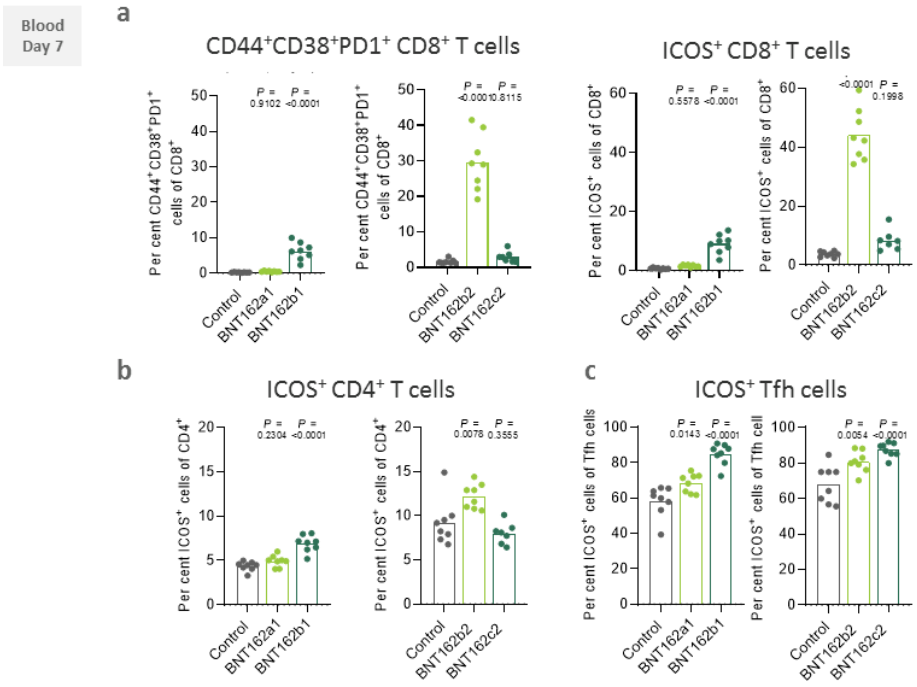

Figure 8: Analysis of T cell activation in the blood of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of blood 7 days after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Buffer treated mice served as control. Cell fractions per mouse are shown by dots; group mean values are indicated by bars.

Phenotypic T- and B-cell analysis dLNs

dLNs were analyzed 12 days (BNT162a1, BNT162b1, BNT162b2) or 27 days (BNT162c2) after vaccination. As shown for the frequency among $CD3^+$ T cells in the blood (Figure 7a), $CD8^+$ T cell counts in the dLNs were significantly elevated in the BNT162b2 group (Figure 9a). $CD4^+$ T cells as well as T_{FH} cells were significantly increased in mice treated with BNT162b1 or BNT162b2 (Figure 9b,c). T_H1 T cell increase was most pronounced in the BNT162b1 (P=0.0134) and BNT162b2 (P=0.0531) groups (Figure 9d).

In line with increased T_{FH} cell counts, B cell numbers were highest in BNT162b1 (P=0.0053) and BNT162b2 (P>0.0001) vaccinated mice (Figure 10a). Among B cells, antibody secreting plasma B cells, class switched B cells and germinal center B cells crucial for affinity maturation of antibodies were significantly expanded (Figure 10b-d). In BNT162a1, BNT162b1 and BNT162b2 groups only, germinal center B cells demonstrated a class switch to IgG1 (BNT162a1, BNT162b1 and BNT162b2) or IgG2a (BNT162b1 and BNT162b2) (Figure 10e,f).

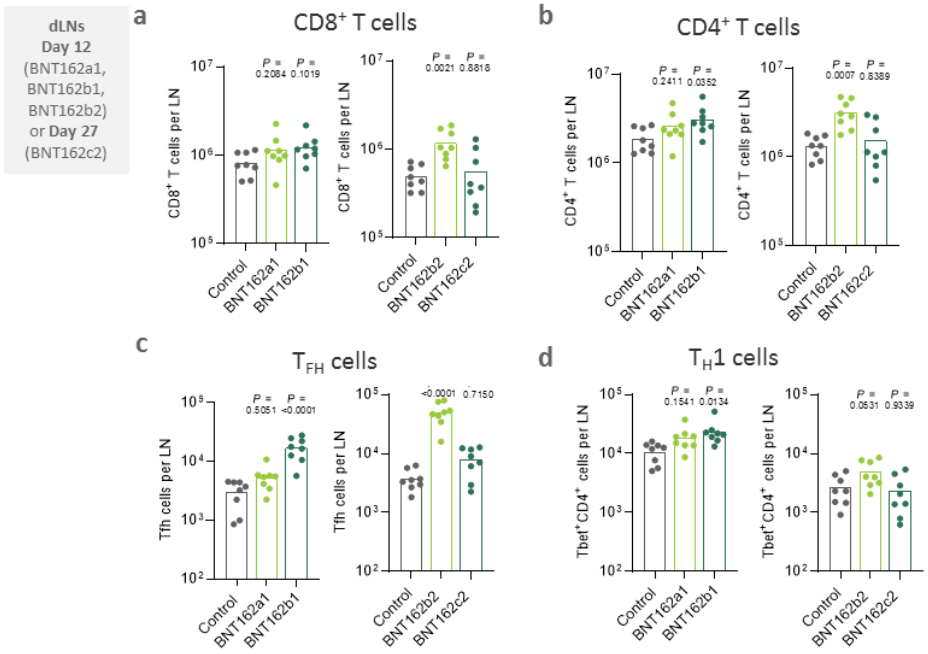

Figure 9: Analysis of T cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of T cells in the dLNs after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

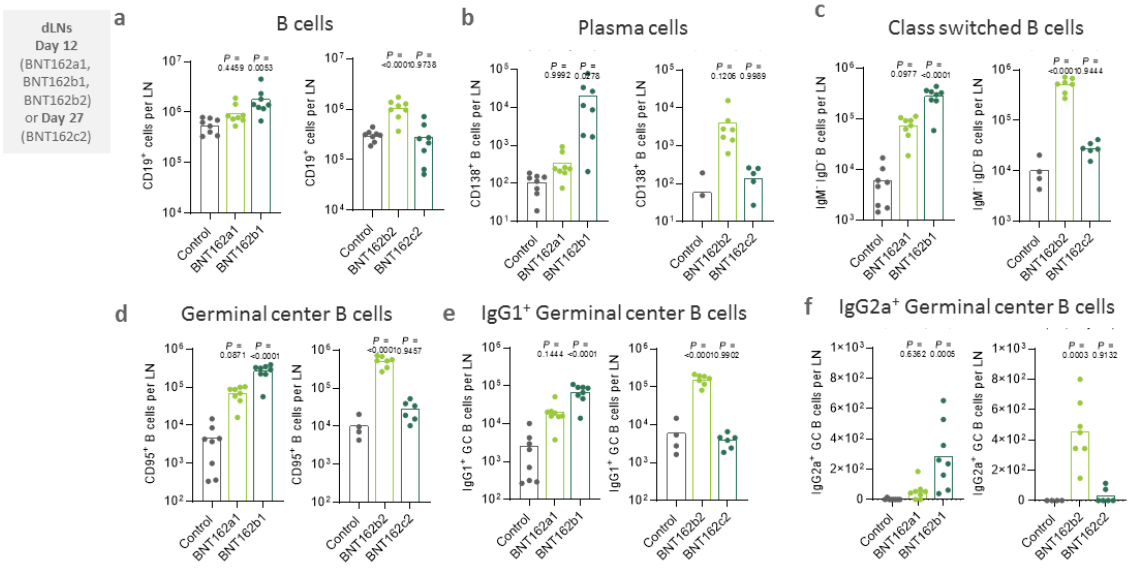

Figure 10: Analysis of B cell counts in the dLNs of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of B cells in the dLNs after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after

vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

Phenotypic T- and B-cell analysis in the spleen

Analysis of T cells and B cells in the spleen revealed similar but less pronounced results compared to blood and dLNs. T_{FH} cells, germinal center B cells and class switched B cells were significantly increased upon BNT162b1 or BNT162b2 vaccination (Figure 11).

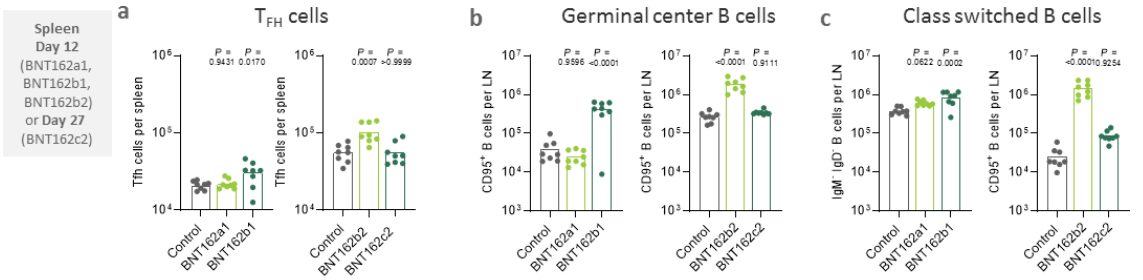

Figure 11: Analysis of T_{FH} and B cell counts in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of T_{FH} cells (**a**), germinal center B cells (**b**) and class switched B cells (**c**) in the spleen after BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccination (N=8 per group). Cells were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Buffer treated mice served as control. Cell counts per mouse are shown by dots; group mean values are indicated by bars.

Functional T-cell analysis in the spleen

Splenocytes were analyzed by intracellular cytokine staining 12 days (BNT162a1, BNT162b1, BNT162b2) or 27 days (BNT162c2) after vaccination, to quantify antigen-specific T cells via flow cytometry. Secretion of IFNγ, IL-2 or TNF was determined in unstimulated or S peptide restimulated samples. Responses without stimulation were subtracted from S peptide stimulated samples from the same mouse and depicted for each treatment group. Cytokine responses in vaccinated animals were compared to buffer treated mice (Control) (Figure 12).

In line with ELISpot data (Figure 6), significant antigen-specific secretion of IFNγ among $CD8^+$ T cells was detectable in splenocytes of BNT162b1, BNT162b2 and BNT162c2 vaccinated animals. $CD8^+$ T cells from BNT162b1 and BNT162b2 vaccinated mice also showed significant release of IL-2 and TNF (Figure 12a). Significant numbers of $CD4^+$ T cells from BNT162b1 vaccinated mice secreted the T_H1 cytokines IFNγ and IL-2, but not the T_H2 cytokine IL-4 (Figure 12b). Although numbers were generally low and the spread between treated groups high, significant antigen-specific secretion of IFNγ among T_{FH} cells was detected in the BNT162b2 group (Figure 12c).

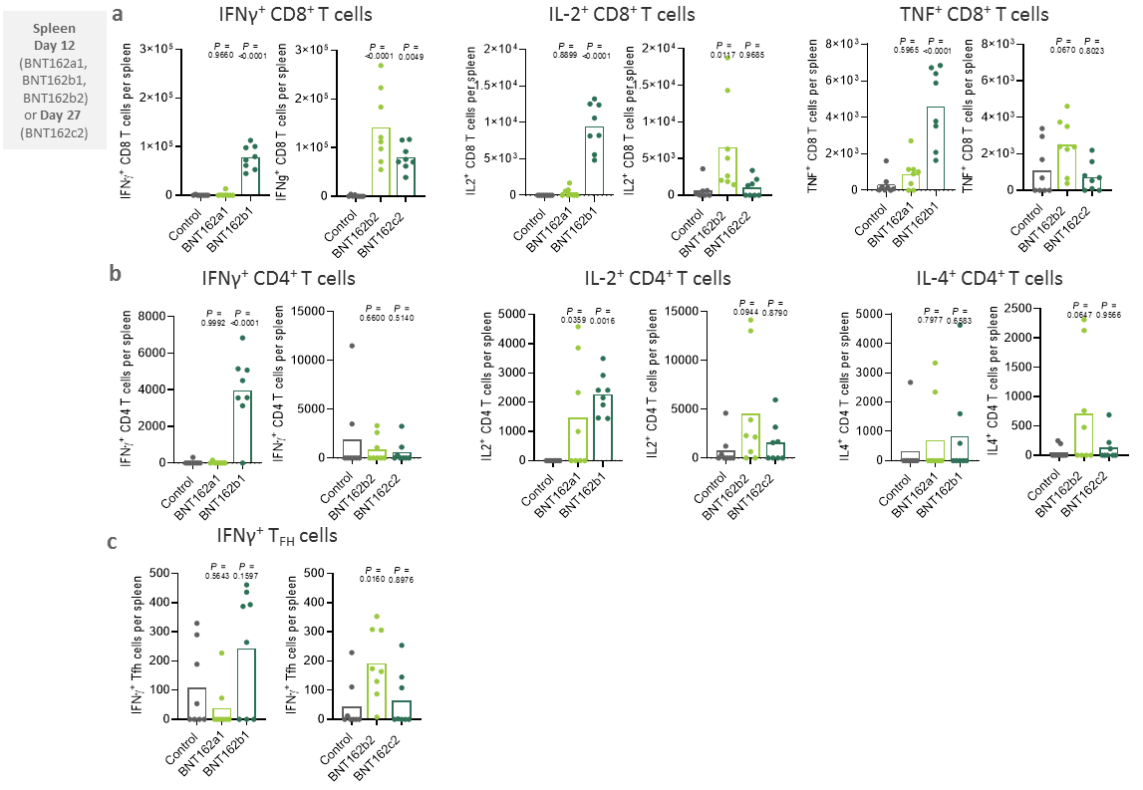

Figure 12: Quantification of cytokine secreting T cells upon S peptide restimulation in the spleen of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Flow cytometry analysis of cytokine secreting $CD8^+$ (**a**), $CD4^+$ (**b**) and T_{FH} cells (**c**) upon S peptide restimulation. Splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes of buffer treated mice served as control. Cytokine positive cell counts per mouse are shown by dots; group mean values are indicated by bars. Values represent S peptide restimulated samples subtracted by unstimulated samples from the same mouse.

In summary, particularly BNT162b1, BNT162b2 and BNT162c2 vaccination mediated a potent T-cell response demonstrated by overall increased T-cell numbers, expression of molecules related to T-cell activation and the production of effector cytokines. Mainly BNT162b1 and BNT162b2 mediated a T_{FH} response in the dLNs, B cell proliferation, and the generation of significant numbers of plasma B cells and germinal center B cells undergoing Ig class switch and affinity maturation.

5.3 Cytokine Multiplex Assay

Complimentary to the analysis of cytokine secretion by IFNγ ELISpot and flow cytometry, spleen and LN cells were restimulated for 48 h with S peptide mixes or without peptide, and the release of cytokines quantified by a bead-based multiplex assay. Buffer treated animals served as control group. Unstimulated samples (cell culture medium) were compared to S peptide restimulated samples and P-values were

determined by two-way ANOVA and Sidak's multiple comparisons test. Detection ranges are provided in Table 20. Raw data including tissues and cytokines not shown in Figure 13 can be found in Table 21 to

Table 32.

Significant antigen-specific release of the T_H1 cytokines IFNγ and IL-2 was observed in the BNT162b1, BNT162b2 and BNT162c2 vaccinated groups (Figure 13a). Splenocytes from BNT162a1 treated mice mediated a significant IL-2 response and a weak IFNγ release in three of eight mice. Highest responses for both cytokines surpassing the upper limit of quantification for IFNγ were found in the BNT162b2 and BNT162c2 groups encoding the full-length S protein. Comparably weak or no secretion of the T_H2 cytokines IL-4 and IL-5 was measured (Figure 13b). Low but significant release of IL-4 and IL-5 was shown for BNT162b2 and BNT162c2. IL-4 but not IL-5 was detected in the supernatant of splenocytes from BNT162b1 vaccinated mice. Besides T_H1 cytokines, high amounts of proinflammatory IL-18 were released in the BNT162b2 and BNT162c2 vaccinated groups, and to lesser extent in the BNT162b1 and BNT162a1 vaccinated groups (Figure 13c). Additional proinflammatory cytokines were significantly elevated, such as GM-CSF (Figure 13d) or IL-6 (not shown), particularly in the BNT162b1, BNT162b2 and BNT162c2 vaccinated groups.

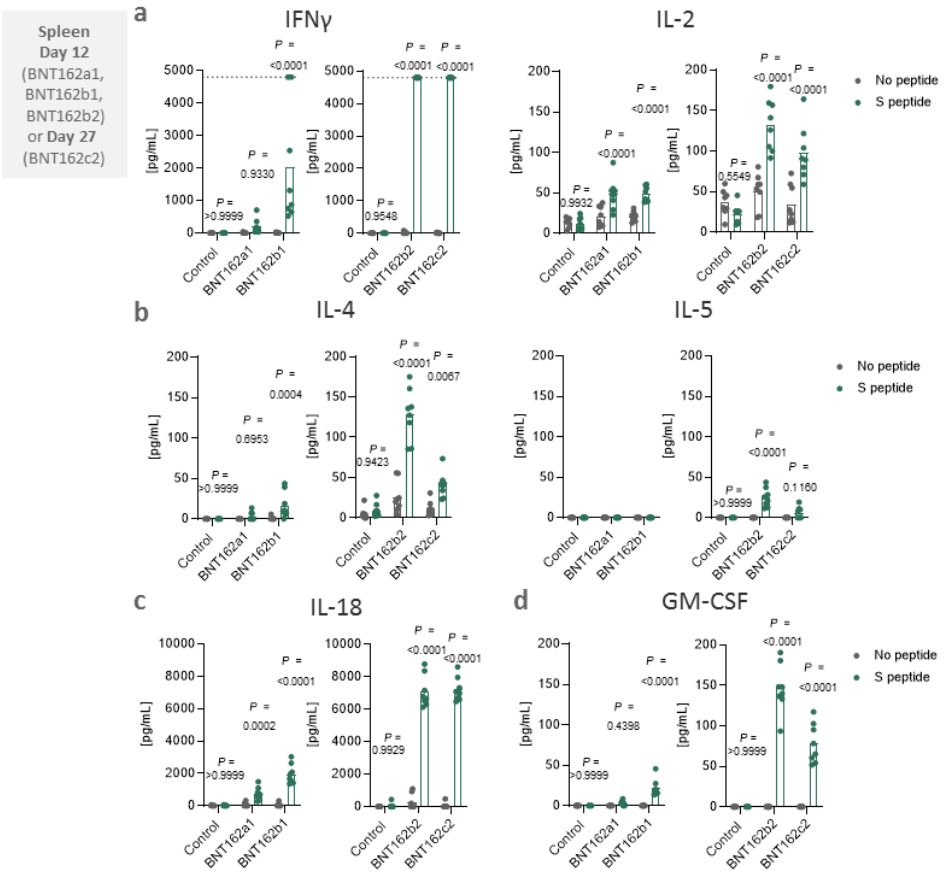

Figure 13: Quantification of cytokine secretion upon S peptide restimulation of splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice

Cytokine multiplex analysis of supernatants of splenocytes upon S peptide restimulation. Splenocytes of BNT162a1, BNT162b1, BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were isolated on day 12 (BNT162a1, BNT162b1, BNT162b2) or day 27 (BNT162c2) after vaccination. Splenocytes of buffer treated mice (N=3) served as control. Dots indicate individual values, group mean values are indicated by bars, horizontal dotted lines indicate the upper limit of detection (ULOQ). Values below the lower limit of quantification (LLOQ) were set to zero. Values above the upper limit of quantification (ULOQ) were set to the ULOQ.

5.4 xCELLigence Cytotoxicity Assay

Isolated $CD8^+$ splenocytes were probed for their capacity to kill CT26 cells electroporated with S RNA (mCorVac#15) and additionally pulsed with S peptide mixes (mCorVac#16). $CD8^+$ T cells stimulated with CT26 cells electroporated with irrelevant RNA served as negative control. Complete tumor cell lysis was modeled by addition of Staurosporin to the S RNA electroporated or S peptide mix loaded CT26 cells. Raw data can be found in Attachment III.

In line with weak antigen-specific cytokine release (Figure 6, Figure 12, Figure 13), no relevant CT26 cell lysis was observed in the BNT162a1 group. For the BNT162b1 vaccinated group, a tendency for cell killing was observed in four out of eight mice (3-2, 3-3, 3-4 and 3-6) given that the Normalized Cell Index of CT26 cells electroporated

with irrelevant RNA was higher than for S RNA electroporated cells (Figure 14). More pronounced tumor cell lysis in eight out of eight mice was observed for splenocytes of mice vaccinated with BNT162b2 or BNT162c2, which encode the full-length S protein (Figure 15). Overall, the detected effects were rather weak and warrant further optimization of the assay. No quantitative and statistical analysis of this dataset was performed.

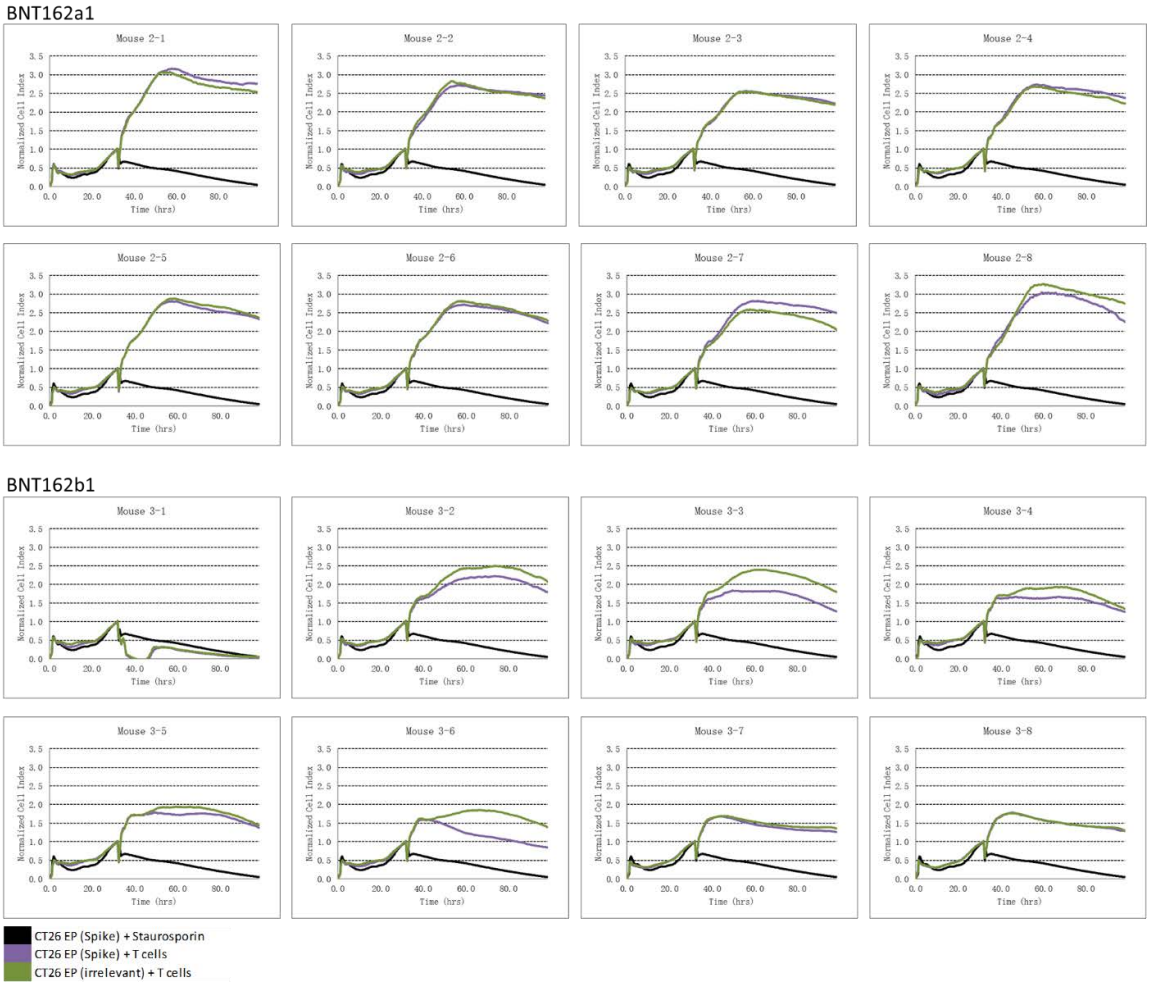

Figure 14: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162a1 or BNT162b1 vaccinated mice (mCorVAC#15).

Splenocytes of BNT162a1 or BNT162b1 vaccinated mice (N=8 per group) were cultured over night with S peptide and recombinant IL-2 and subsequently CD8⁺ cells were isolated via magnetic bead based separation (MACS). CT26 cells electroporated with S RNA or irrelevant RNA were cultured in xCELLigence plates for 24 h prior to addition of isolated CD8⁺ T cells. CT26 cell numbers were quantified via impedance measurement (Normalized Cell Index, higher values indicate more viable CT26 cells, normalization was performed at the time point of T cell addition). Staurosporin treatment modeled complete tumor cell lysis. CT26 cells transfected with irrelevant RNA served as negative control. Depicted is the Normalized Cell Index over time for individual mice.

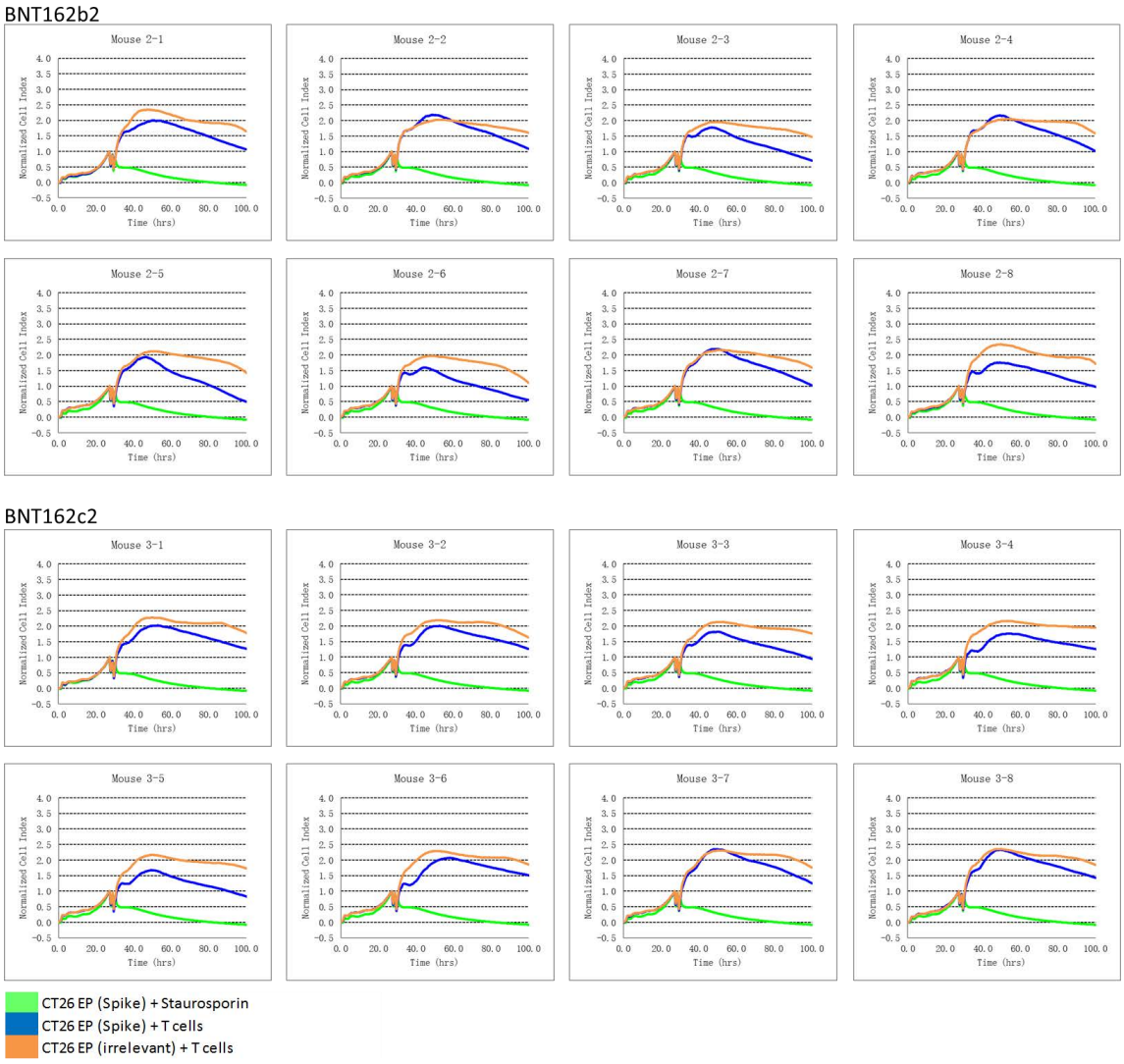

Figure 15: Cytotoxicity towards S protein expressing CT26 cells by CD8⁺ splenocytes from BNT162b2 or BNT162c2 vaccinated mice (mCorVAC#16).

Splenocytes of BNT162b2 or BNT162c2 vaccinated mice (N=8 per group) were cultured over night with S peptide and recombinant IL-2 and subsequently CD8⁺ cells were isolated via magnetic bead based separation (MACS). CT26 cells electroporated with S RNA or irrelevant RNA were cultured in xCELLigence plates for 24 h. Prior to addition of isolated CD8⁺ T cells, S RNA transfected CT26 cells were pulsed with S peptide. CT26 cell numbers were quantified via impedance measurement (Normalized Cell Index, higher values indicate more viable CT26 cells, normalization was performed at the time point of T cell addition). Staurosporin treatment modeled complete tumor cell lysis. CT26 cells transfected with irrelevant RNA served as negative control. Depicted is the Normalized Cell Index over time for individual mice.

6 CONCLUSION

This study aimed at characterizing T- and B-cell responses induced by the COVID-19 vaccine candidates BNT162a1, BNT162b2, BNT162b1 and BNT162c2 in detail.

Overall, the results of the different assay types pointed towards similar conclusions, highlighting the validity of the obtained data. IFNγ ELISpot assay, flow cytometry analysis and multiplexed quantification of cytokines suggested that particularly BNT162b1, BNT162b2 and BNT162c2 vaccination induced a potent T-cell response demonstrated by overall increased T-cell numbers, expression of molecules related to T-cell activation and the potential of T cells to produce cytokines. T-cell responses showed primarily a T_H1 phenotype with increased numbers of T-bet$^+$ CD4$^+$ T cells (mainly BNT162b1 and BNT162b2) and high secretion of T_H1 type cytokines (IFNγ, IL-2, TNF) and low secretion of T_H2 type cytokines (IL-4, IL-5). Mainly BNT162b1 and BNT162b2 mediated a T_{FH} response in the dLNs, B cell proliferation and the generation of significant numbers of antibody producing plasma B cells and germinal center B cells undergoing Ig class switch and affinity maturation.

The results of this study are in agreement with prior studies investigating the number of IFNγ specific T cells by ELISpot and IgG titers by ELISA 28 days after vaccination (R-20-0040, R-20-0042, R-20-0053, R-20-0085). Similarly to this study, responses of BNT162b1 and BNT162b2 were much stronger compared to BNT162a1 in those studies.

Since the kinetics of expression for the vaccine encoded protein of BNT162c2 differs from the other three vaccine candidates, the analysis time point was set on day 27 instead of day 12 after vaccination. It is possible that the selected time point was suboptimal and missed the peak expansion of lymphocytes. BNT162c2 induced a potent T-cell response (IFNγ ELISpot, intracellular cytokine staining by flow cytometry and multiplexed protein quantification) including the highest T_{FH} cell responses amongst all tested candidates in the blood on day 7 after treatment. However, in the dLNs on day 27 after vaccination, the impact on T_{FH} cells and B cells was weak to undetectable. Effects of BNT162c2 on both T and B cells might be stronger when analyzed at an earlier time point. Direct comparison of BNT162c2 to BNT162a1, BNT162b2 or BNT162b1 is therefore difficult and might underestimate the potential of BNT162c2.

Due to the prominent induction of both T- and B-cell responses, these results particularly support further clinical evaluation of the COVID-19 vaccine candidates BNT162b1 and BNT162b2 and warrant further evaluation of BNT162c2.

7 DOCUMENT HISTORY

First version / no change.

8 REFERENCES

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9 APPENDIX

9.1 Animal Monitoring

9.2 Animal Monitoring - Observations

Table 14: Parameters for experimental animal monitoring (single animal assessment)

The table is separated in immediate euthanasia criteria (end of experiment) and criteria, which, solitarily observed, do not lead to an immediate termination, but result in higher monitoring frequency of re-assessment. BCS, body conditioning score.

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate accumulated parameters**	**Immediate euthanasia criteria**
1	Bodyweight [b]. Take into account BCS [c]	Body weight loss >5–10%, or BCS transition 3 to 2	Body weight loss >15-20%, or BCS 2
2	Activity	Moderate deviation from normal or unusual behavior (e.g. limited, reduced or hyperactive movements)	Immobility, very slow movements (high grade of lethargy), self-isolation
3	Appearance (condition) of fur & eyes	Fur defects/ grooming malfunction (reduced or exaggerated grooming). Moderate orbital tightening.	Distinct scruffy fur, strongly neglected grooming. Eye lids narrowed, eyes closed and sticky.
4	Body cavities & body fluids	Slight to moderate damp & sticky cavities	Clinical signs of disease (diarrhea, distinct sticky)
5	Body temperature & blood circulation ears	-	Body temperature low, ears appear white and hardly noticeable blood vessels

		Observation (if applicable, categorize [a]):	
Code	**Parameter**	**Renew assessment within < 24 h. Attention: evaluate accumulated parameters**	**Immediate euthanasia criteria**
6	Posture	Moderate deviation of normal physiological posture i.e., short pause in hunched posture	Abnormal posture, hunched, abnormally stretched (belly touches ground) or cramps
7	Reaction to stimulus [d]	Delayed reaction to unconditioned stimulus, moderate deviation from normal behavior (e.g. slight to moderate apathy)	Abnormal (distinct delayed reaction to unconditioned stimulus). Winding and enduring sound utterance ("pain"), aggressiveness to touch
8	Automutilation	-	Noticable burden, i.e. missing extremities, continuous nibbling, biting and gnawing, open wounds
9	Bites (tail, vibrissae, reproductive organs…), other wounds	Open and bleeding wounds (take care of wounds and separate from others)	Noticable burden, i.e. inflamed wounds
10	Respiration frequency	Moderate deviation of spontaneous breathing (normal respiration frequency)	High frequency, any sign of dyspnea, gasping, flat stretched posture in combination with strongly retracting flanks
11	Motor function	Weak, loose grip (cage grid)	Staggering, circular movement, missing grasp
12	Other abnormalities [e]	-	-

a Categories: NAD, no abnormality detected; +, slight; ++, moderate; +++, distinct.
b Calculate ratio bodyweight start of experiment/bodyweight monitoring day.
c According to Ullman-Culleré and Foltz 1999.
d Unconditioned = Stimulus to force a reaction e.g. normal background noise, tapping the cage and normal handling procedure e.g. tilt and turns of the cage.
e Description of abnormality (or abnormalities) on monitoring sheet.

Table 15: Record of body weights of mCorVAC#15 animals during study

						Bodyweight (grams)						
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	20.4	20.3	20.5	20.6	20.3	20.8	20.6
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	22.1	22.6	22.5	22.3	22.4	23.5	22.7
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	20.9	21.1	20.9	20.8	20.9	21.6	21.3
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	21.7	21.5	21.4	21.0	21.2	22.5	22.1
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	19.6	19.8	20.2	20.4	20.7	20.5	21.2
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	20.9	20.7	21.2	21.0	21.6	20.9	21.3
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	19.7	19.5	19.5	19.3	19.9	20.3	19.9
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	18.9	18.6	18.3	18.4	19.0	18.9	18.9
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	20.9	20.6	20.9	21.2	20.8	21.1	21.2
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	21.3	19.3	20.2	22.7	21.4	21.1	20.7
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	23.2	20.5	21.9	22.5	22.4	22.9	22.9
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	19.8	18.9	20.0	20.8	20.3	21.0	20.7
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	22.5	20.9	21.3	21.7	21.6	21.7	21.6
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	20.9	19.2	20.6	21.6	20.8	20.8	20.9
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	21.8	21.1	21.5	22.1	21.8	21.5	22.1
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	22.7	20.6	21.8	22.5	22.5	22.2	22.8
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	19.3	18.2	18.9	19.0	18.9	18.9	18.9
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	21.1	21.6	20.6	21.1	21.2	21.9	21.1
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	20.3	19.3	20.2	20.5	20.8	20.3	20.2
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	22.9	22.0	23.0	23.4	23.3	22.9	22.3
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	21.1	21.0	21.7	21.7	22.6	23.1	23.3
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	19.9	18.9	19.3	19.7	19.2	19.9	19.2
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	22.1	21.0	22.3	22.3	20.8	22.1	21.9
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	20.6	19.8	21.1	21.4	22.1	21.1	21.3

Table 16: Record of animal monitoring during CorVac#15 study

12: swelling of injection site muscle

						Animal Monitoring - Observations						
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	NAD	3+;12+	12+	NAD	12+	NAD	NAD
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	NAD	3+;12+	12+	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	NAD	12+	NAD	NAD
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	12+	NAD	NAD
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	NAD	3+;12+	12+	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	NAD	3+;12+	12+	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12+	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	NAD	NAD	NAD
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12++	12+	NAD	NAD
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12+	12+	NAD	NAD	NAD

Table 17: Record of body weights of CorVac#16 animals during study

n/a: not available (Treatment group 1+2: no weight measurement performed as treatment had just occurred [day 15]; Treatment group 3: Weekly weight measurement sufficient)

						Bodyweight (grams)												
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22
SBIO-15337	BIO-LO78	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.1	20.8	20.8	21.1	21.5	21.0
SBIO-15337	BIO-LO79	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.3	21.2	20.9	21 0	21.9	21.9
SBIO-15337	BIO-LO80	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.0	20.2	20.2	20 3	21.2	20.7
SBIO-15337	BIO-LO81	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.5	22.4	21.9	21 9	23.1	22.7
SBIO-15338	BIO-LO82	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	22.4	22.3	22.1	22.1	22.6
SBIO-15338	BIO-LO83	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.2	20.5	20.6	20.6	20.7	21.1
SBIO-15338	BIO-LO84	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	20.5	21.1	20.8	21 2	21.7	20.8
SBIO-15338	BIO-LO85	BALB/cJRj	f	03 03 20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	22.6	22.3	21 9	22.6	22.3
SBIO-15339	BIO-LO86	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.5	21.7	22.6	23 2	22.9	23.1
SBIO-15339	BIO-LO87	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.1	21.3	21.9	22 5	23.2	22.1
SBIO-15339	BIO-LO88	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.7	20.9	21.6	21.6	22.2	22.1
SBIO-15339	BIO-LO89	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.5	21.2	22.6	22.7	23.2	22.7
SBIO-15340	BIO-LO90	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.9	20.3	20.5	21.1	21.4	21.1
SBIO-15340	BIO-LO91	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	21.1	20.3	20.4	22.7	21.1	20.6
SBIO-15340	BIO-LO92	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	22.4	21.7	23.9	23 8	23.8	22.5
SBIO-15340	BIO-LO93	BALB/cJRj	f	03 03 20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	23.3	21.7	22.4	20 9	22.7	22.2
SBIO-15341	BIO-LO94	BALB/cJRj	f	03 03 20	3	21 8	20.8	21 5	22.1	21.9	22.1	22.1	n/a	23.4	n/a	n/a	n/a	22.8
SBIO-15341	BIO-LO95	BALB/cJRj	f	03 03 20	3	20 8	19.3	20 3	21.2	20.6	21.1	21.5	n/a	21.7	n/a	n/a	n/a	22.1
SBIO-15341	BIO-LO96	BALB/cJRj	f	03 03 20	3	22.4	20.1	21.4	22.5	22.1	22.2	22.1	n/a	23.3	n/a	n/a	n/a	22.8
SBIO-15341	BIO-LO97	BALB/cJRj	f	03 03 20	3	19.1	17.6	17 8	19.1	18.5	19.4	20.5	n/a	19.5	n/a	n/a	n/a	19
SBIO-15342	BIO-LO98	BALB/cJRj	f	03 03 20	3	18.7	17.2	18 0	18.6	18.5	18.8	18.2	n/a	20.1	n/a	n/a	n/a	20.6
SBIO-15342	BIO-LO99	BALB/cJRj	f	03 03 20	3	20 5	19.2	20.4	21.2	21.1	21.7	20.9	n/a	22.1	n/a	n/a	n/a	21.5
SBIO-15342	BIO-LP00	BALB/cJRj	f	03 03 20	3	19.6	17.6	19.1	19.8	19.9	19.9	19.9	n/a	22.3	n/a	n/a	n/a	22.8
SBIO-15342	BIO-LP01	BALB/cJRj	f	03 03 20	3	18.1	16.8	17.4	17.9	18.2	18.2	18.1	n/a	19.8	n/a	n/a	n/a	19

Table 18: Record of animal monitoring during CorVac#16 study

12: swelling of injection site muscle

n/a: not available (no weight measurement performed as treatment had just occurred [day 15])

						Animal Monitoring - Observations												
Cage	Mouse ID	Strain	Gender	Date of birth	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 15	Day 16	Day 17	Day 18	Day 19	Day 22
SBIO-15337	BIO-LO78	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO79	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO80	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15337	BIO-LO81	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO82	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO83	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO84	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15338	BIO-LO85	BALB/cJRj	f	03.03.20	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15339	BIO-LO86	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12+	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO87	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	12+	NAD	NAD
SBIO-15339	BIO-LO88	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	NAD	NAD	NAD
SBIO-15339	BIO-LO89	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO90	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12+	12+	NAD	NAD
SBIO-15340	BIO-LO91	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	NAD	NAD	NAD
SBIO-15340	BIO-LO92	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12+	12+	NAD	NAD	NAD
SBIO-15340	BIO-LO93	BALB/cJRj	f	03.03.20	2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	NAD	12++	12++	12+	NAD	NAD
SBIO-15341	BIO-LO94	BALB/cJRj	f	03.03.20	3	NAD	3++;12++	3++;12++	3++;3++	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO95	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3+;12++	3+; 12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO96	BALB/cJRj	f	03.03.20	3	NAD	3++;12++	3+;12++	3+;12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15341	BIO-LO97	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3++;12++	3+; 12++	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO98	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LO99	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	12+	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LP00	BALB/cJRj	f	03.03.20	3	NAD	3+;12++	12++	NAD	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
SBIO-15342	BIO-LP01	BALB/cJRj	f	03.03.20	3	NAD	3++;12+++	3+;12+++	3+;12+	12++	12+	NAD	NAD	NAD	NAD	NAD	NAD	NAD

9.3 ELISpot – Raw data

Table 19: ELISpot raw data.

TNTC, too numerous to count (these values are set to 1,500 in Figure 6). Thousands were not separated by commas.

		Stimulation (well 1 | well 2)							
Group	**Mouse**	**No peptide**		**S peptide**		**Control RNA**		**S RNA**	
Control (mCorVac#15)	1	2	1	4	3	0	1	3	1
	2	3	2	2	2	6	11	2	1
	3	3	1	8	11	7	3	6	3
	4	5	6	6	4	2	4	3	4
	5	6	4	11	15	5	9	6	3
	6	6	5	9	13	5	7	4	5
	7	3	5	8	14	12	14	11	6
	8	8	4	18	15	5	6	4	1
BNT162a1	1	13	13	118	127	7	6	57	63
	2	12	9	128	148	12	7	98	101
	3	23	17	75	86	5	9	39	40
	4	14	21	51	48	5	5	38	34
	5	20	18	87	107	13	9	43	51
	6	17	23	132	156	11	22	48	84
	7	15	14	69	65	7	3	38	41
	8	18	42	96	121	13	18	64	67
BNT162b1	1	42	44	658	645	19	21	676	615
	2	11	16	456	440	21	14	399	322
	3	21	23	889	977	8	9	1124	1218
	4	26	21	871	918	11	12	779	751
	5	22	26	873	834	15	9	841	881
	6	33	16	733	746	12	12	758	842
	7	16	24	861	837	16	11	825	702
	8	17	18	837	772	9	8	628	598
Control (mCorVac#16)	1	21	9	7	57	12	11	8	11
	2	4	15	7	28	28	31	18	16
	3	13	5	12	23	11	7	6	9

		Stimulation (well 1 | well 2)							
Group	**Mouse**	**No peptide**		**S peptide**		**Control RNA**		**S RNA**	
	4	19	19	26	38	4	9	6	7
	5	8	9	19	31	8	8	7	2
	6	22	13	26	28	19	12	11	17
	7	17	15	21	24	17	20	12	13
	8	14	11	37	62	12	15	16	26
BNT162b2	1	6	14	1267	1296	13	13	1674	1628
	2	20	17	1196	1147	15	20	1281	1268
	3	17	20	1503	1404	39	37	1278	1117
	4	11	13	1311	1289	20	17	1226	1324
	5	21	21	911	881	23	12	1171	1391
	6	15	25	1126	1173	11	13	1143	1427
	7	9	14	1128	1096	15	16	1435	1334
	8	33	24	TNTC	TNTC	59	62	TNTC	TNTC
BNT162c2	1	7	6	1315	1328	9	18	1348	1263
	2	11	13	1315	1328	24	4	1222	1089
	3	7	5	1328	1267	12	14	1351	1206
	4	21	16	877	1135	24	15	1188	1173
	5	12	9	1371	1199	19	11	1504	1246
	6	6	14	1025	786	5	20	1143	1232
	7	11	15	1218	1132	21	22	1034	911
	8	4	6	1275	1054	13	6	973	1092

9.4 Cytokine multiplex analysis – Assay detection ranges

Table 20: Detection ranges of the ProcartaPlex immunoassay for mCorVAC#15 and mCorVAC#16.

Depicted are lower limt of quantification (LLOQ) and upper limit of quantification (ULOQ) for each analyte. LN, lymph node. SP, spleen.

[pg/mL]	IFNγ	IL-12p70	IL-13	IL-1β	IL-2	IL-4	IL-5	IL-6	TNFα	GM-CSF	IL-18
mCorVAC#15 (SP, LN)	1.1-4,800	1.5-409.3	2.1-8,650	1-4,350	1.2-5,250	4.8-4,950	1.9-8,000	4.7-19,500	2.8-731.2	2.4-9,950	50.5-207,000
mCorVAC#16 Plate 1 (SP)	1.1-4,800	1.5-102.3	2.1-2,162.5	1-1,087.5	1.2-1,312.5	1.2-4,950	7.8-2,000	4.7-4,875	2.8-731.2	9.7-2,487.5	202.1-51,750
mCorVAC#16 Plate 2 (LN)	1.1-4,800	1.5-102.3	2.1-8,650	1-4,350	1.2-5,250	1.2-4,950	1.9-8,000	4.7-19,500	2.8-731.2	2.4-9,950	202.1-51,750

9.5 Cytokine multiplex analysis – Raw data and calculated data

Table 21: Cytokine raw data and calculated data for mCorVAC#15, part 1 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	Medium	SP	187,5	1,26	1,26	11	<=0	0	24	<=0	0	17	0,28	0	754	14,82	14,82
3	1	2	Medium	SP	487	3,99	3,99	13	<=0	0	25	<=0	0	18	0,3	0	583	11,34	11,34
4	1	3	Medium	SP	25	0,24	0	10	<=0	0	21	<=0	0	17	0,28	0	152	2,85	2,85
5	1	4	Medium	SP	54	0,39	0	11	<=0	0	21,5	<=0	0	18	0,3	0	813,5	16,05	16,05
6	1	5	Medium	SP	27	0,25	0	9	<=0	0	21	<=0	0	16	0,26	0	333	6,38	6,38
7	1	6	Medium	SP	118	0,77	0	11	<=0	0	27	0,03	0	13	0,2	0	1012,5	20,24	20,24
8	1	7	Medium	SP	46,5	0,35	0	11	<=0	0	59,5	1,34	0	14	0,22	0	915	18,17	18,17
9	1	8	Medium	SP	124	0,81	0	10	<=0	0	28	0,07	0	12	0,18	0	737,5	14,48	14,48
10	2	1	Medium	SP	1249,5	14,42	14,42	20	<=0	0	44	0,70	0	19	0,32	0	1778	37,67	37,67
11	2	2	Medium	SP	165,5	1,10	1,10	14,5	<=0	0	41,5	0,60	0	15	0,24	0	539	10,45	10,45
12	2	3	Medium	SP	219	1,51	1,51	13	<=0	0	30	0,14	0	21	0,36	0	406,5	7,82	7,82
13	2	4	Medium	SP	50	0,37	0	11	<=0	0	33	0,26	0	16	0,26	0	470	9,08	9,08
14	2	5	Medium	SP	2466	40,87	40,87	30	<=0	0	123	4,11	4,11	26	0,47	0	1123	22,62	22,62
15	2	6	Medium	SP	455	3,66	3,66	12	<=0	0	62	1,44	0	16	0,26	0	730	14,32	14,32
16	2	7	Medium	SP	162,5	1,08	0	12	<=0	0	28	0,07	0	17,5	0,29	0	1605	33,53	33,53
17	2	8	Medium	SP	327	2,42	2,42	17	<=0	0	31	0,18	0	19	0,32	0	1560	32,47	32,47
18	3	1	Medium	SP	2160,5	33,01	33,01	36	<=0	0	43	0,66	0	45,5	0,91	0	1498,5	31,04	31,04
19	3	2	Medium	SP	446	3,57	3,57	15	<=0	0	33	0,26	0	18	0,30	0	1318	26,93	26,93
20	3	3	Medium	SP	380,5	2,92	2,92	14	<=0	0	48	0,86	0	20	0,34	0	755	14,84	14,84
21	3	4	Medium	SP	265	1,88	1,88	15	<=0	0	63	1,49	0	20	0,34	0	657,5	12,84	12,84
22	3	5	Medium	SP	154	1,02	0	16,5	<=0	0	98,5	3,02	3,02	18	0,30	0	1112	22,38	22,38
23	3	6	Medium	SP	128	0,84	0	12	<=0	0	46	0,78	0	15	0,24	0	1013	20,25	20,25
24	3	7	Medium	SP	77	0,52	0	11	<=0	0	26	0,00	0	16	0,26	0	1116	22,47	22,47
25	3	8	Medium	SP	347	2,61	2,61	14	<=0	0	115	3,75	3,75	18	0,30	0	902	17,90	17,90

Table 22: Cytokine raw data and calculated data for mCorVAC#15, part 2 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	Medium	SP	37	<=0	0	10	<=0	0	21	2,36	0	36	0,90	0	9	0,31	0	25	16,07	0
3	1	2	Medium	SP	48	<=0	0	10	<=0	0	27	3,34	0	30	0,72	0	12	0,42	0	48	61,82	61,82
4	1	3	Medium	SP	24	<=0	0	11	<=0	0	24,5	2,93	0	26	0,60	0	6	<=0	0	15	<=0	0
5	1	4	Medium	SP	22	<=0	0	10	<=0	0	23	2,68	0	27	0,63	0	9	0,31	0	14,5	<=0	0
6	1	5	Medium	SP	12	<=0	0	11	<=0	0	18	1,89	0	27	0,63	0	7	0,24	0	15	<=0	0
7	1	6	Medium	SP	15	<=0	0	10	<=0	0	22,5	2,60	0	25	0,58	0	7	0,24	0	22	9,40	0
8	1	7	Medium	SP	24	<=0	0	15	<=0	0	22	2,52	0	29	0,69	0	15,5	0,55	0	15	<=0	0
9	1	8	Medium	SP	25	<=0	0	11	<=0	0	22	2,52	0	33	0,81	0	9,5	0,33	0	22	9,40	0
10	2	1	Medium	SP	131,5	0,82	0	12	<=0	0	81	13 37	13,37	43	1,11	0	21	0,75	0	108	169,05	169,05
11	2	2	Medium	SP	153	1,18	0	12,5	<=0	0	70	11,20	11,20	32	0,78	0	10	0,35	0	25	16,07	0
12	2	3	Medium	SP	148	1,09	0	10	<=0	0	56,5	8,61	8,61	31	0,75	0	11	0,39	0	29	24,53	0
13	2	4	Medium	SP	164	1,37	0	12	<=0	0	55	8 33	8,33	23	0,52	0	9	0,31	0	16	<=0	0
14	2	5	Medium	SP	149	1,11	0	14,5	<=0	0	413,5	92,66	92,66	909	35,52	35,52	24	0,85	0	200,5	324,27	324,27
15	2	6	Medium	SP	44	<=0	0	16,5	<=0	0	48	7,03	7,03	32	0,78	0	11	0,39	0	44	54,22	54,22
16	2	7	Medium	SP	111	0,5	0	11	<=0	0	62	9,65	9,65	42,5	1,09	0	21	0,75	0	24	13,88	0
17	2	8	Medium	SP	111	0,5	0	10	<=0	0	78	12,77	12,77	40	1,02	0	14	0,50	0	36	38,68	0
18	3	1	Medium	SP	119	0,62	0	12	<=0	0	762	192,57	192,57	1697	77,07	77,07	27	0,96	0	189	305,29	305,29
19	3	2	Medium	SP	83,5	0,11	0	12	<=0	0	59	9,08	9,08	40	1,02	0	22	0,78	0	44	54,22	54,22
20	3	3	Medium	SP	303	4,01	0	11	<=0	0	99,5	17,13	17,13	36	0,90	0	17	0,60	0	39	44,57	0
21	3	4	Medium	SP	275	3,44	0	12,5	<=0	0	157	29,48	29,48	32	0,78	0	13	0,46	0	32	30,67	0
22	3	5	Medium	SP	385	5,72	5,72	29	0,43	0	154	28,82	28,82	33	0,81	0	13	0,46	0	26	18,22	0
23	3	6	Medium	SP	153,5	1,19	0	16,5	<=0	0	107	18,68	18,68	28	0,66	0	15	0,53	0	22	9,40	0
24	3	7	Medium	SP	77	0,03	0	11	<=0	0	45	6,48	6,48	23	0,52	0	11	0,39	0	16	<=0	0
25	3	8	Medium	SP	195	1,92	0	10,5	<=0	0	137	25,08	25,08	38	0,96	0	24	0,85	0	37	40,65	0

Table 23: Cytokine raw data and calculated data for mCorVAC#15, part 3 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
26	1	1	S peptide	SP	386,5	2,98	2,98	12	<=0	0	24	<=0	8650	19	0,32	0	610	11,88	11,88
27	1	2	S peptide	SP	75	0,51	0	13	<=0	0	39	0,50	0	16	0,26	0	955	19,01	19,01
28	1	3	S peptide	SP	178	1,19	1,19	10	<=0	0	30	0,14	0	15	0,24	0	246,5	4,69	4,69
29	1	4	S peptide	SP	35	0,29	0	11	<=0	0	19	<=0		16	0,26	0	190,5	3,60	3,60
30	1	5	S peptide	SP	203	1,38	1,38	9,5	<=0	0	25	<=0		15	0,24	0	365	7,01	7,01
31	1	6	S peptide	SP	94	0,62	0	11	<=0	0	20,5	<=0		13	0,20	0	321	6,14	6,14
32	1	7	S peptide	SP	586,5	5,09	5,09	12	<=0	0	21	<=0		11	0,16	0	885	17,54	17,54
33	1	8	S peptide	SP	318	2,34	2,34	14	<=0	0	23	<=0		13	0,20	0	1203,5	24,38	24,38
34	2	1	S peptide	SP	4966,5	149,75	149,75	35	<=0	0	266	10,75	10,75	27	0,49	0	2348	52,26	52,26
35	2	2	S peptide	SP	5474	186,09	186,09	32	<=0	0	224,5	8,78	8,78	24	0,42	0	1414	29,10	29,10
36	2	3	S peptide	SP	4423,5	117,49	117,49	30	<=0	0	336	14,14	14,14	30	0,56	0	1907	40,84	40,84
37	2	4	S peptide	SP	2160	33,00	33,00	21	<=0	0	107	3,40	3,40	17	0,28	0	1130,5	22,78	22,78
38	2	5	S peptide	SP	7059	356,73	356,73	47,5	<=0	0	682,5	31,94	31,94	32	0,60	0	2363,5	52,68	52,68
39	2	6	S peptide	SP	8699,5	707,98	707,98	62	<=0	0	1080,5	54,24	54,24	31	0,58	0	3508	87,45	87,45
40	2	7	S peptide	SP	2327	37,18	37,18	21	<=0	0	134,5	4,62	4,62	19	0,32	0	2460	55,32	55,32
41	2	8	S peptide	SP	3945	93,72	93,72	29	<=0	0	178	6,61	6,61	22	0,38	0	2176	47,70	47,70
42	3	1	S peptide	SP	12251	5435,41	4800	113	0,53	0	1666	90,58	90,58	53	1,09	1,09	2323	51,59	51,59
43	3	2	S peptide	SP	11207	2540,72	2540,72	67	<=0	0	446,5	19,64	19,64	32	0,60	0	1969,5	42,40	42,40
44	3	3	S peptide	SP	13878	55904,59	4800	123	0,67	0	2112,5	121,31	121,31	50	1,02	1,02	2650	60,65	60,65
45	3	4	S peptide	SP	8838	752,35	752,35	79,5	0,05	0	1314	68,22	68,22	34,5	0,66	0	2477	55,79	55,79
46	3	5	S peptide	SP	10020	1309,58	1309,58	77,5	0,02	0	1206	61,67	61,67	35	0,67	0	1807	38,38	38,38
47	3	6	S peptide	SP	7982	521,41	521,41	55	<=0	0	882	42,87	42,87	27	0,49	0	1849,5	39,42	39,42
48	3	7	S peptide	SP	9172,5	873,94	873,94	58,5	<=0	0	648	30,09	30,09	28	0,51	0	1861	39,70	39,70
49	3	8	S peptide	SP	8488	646,01	646,01	59	<=0	0	1148	58,21	58,21	29	0,53	0	2611	59,54	59,54
52	1	6	PMA Iono	SP	3238	65,07	65,07	338	3,83	3,83	10885	3220,14	3220,14	82	1,81	1,81	16603	5,85E+07	5250
51	1	7	PMA Iono	SP	3585	78,26	78,26	349,5	4,01	4,01	11523	4700,47	4700,47	95	2,15	2,15	17179,5	5,85E+07	5250
50	1	8	PMA Iono	SP	3246	65,35	65,35	319	3,55	3,55	10442	2593,95	2593,95	67	1,43	1,43	16984	5,85E+07	5250
60	2	5	PMA Iono	SP	4643	129,80	129,80	324	3,62	3,62	10730	2975,64	2975,64	118	2,75	2,75	15930	9,10E+04	5250
59	2	6	PMA Iono	SP	4585	126,45	126,45	371,5	4,35	4,35	13072	93260,89	8650	115,5	2,69	2,69	17962	5,85E+07	5250
58	2	7	PMA Iono	SP	5308	173,46	173,46	306,5	3,36	3,36	10521,5	2691,16	2691,16	84,5	1,87	1,87	18176,5	5,85E+07	5250
66	3	1	PMA Iono	SP	4589,5	126,71	126,71	304,5	3,33	3,33	11181	3790,84	3790,84	86	1,91	1,91	16344	1,67E+06	5250
67	3	4	PMA Iono	SP	3133	61,38	61,38	348,5	3,99	3,99	12338	9811,27	8650	105	2,41	2,41	16468,5	5,85E+07	5250
68	3	7	PMA Iono	SP	4499	121,62	121,62	334	3,77	3,77	11511	4662,32	4662,32	100,5	2,29	2,29	17556	5,85E+07	5250

Table 24: Cytokine raw data and calculated data for mCorVAC#15, part 4 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
26	1	1	S pep ide	SP	88	0,16	0	9	<=0	0	32	4,18	0	33	0,81	0	11	0,39	0	41	48,45	0
27	1	2	S pep ide	SP	252	2,99	0	10	<=0	0	85	14,17	14,17	36	0,90	0	10	0,35	0	18	<=0	0
28	1	3	S pep ide	SP	22	<=0	0	8,5	<=0	0	28	3,51	0	23	0,52	0	9	0,31	0	27	20,34	0
29	1	4	S pep ide	SP	16,5	<=0	0	10	<=0	0	17,5	1,81	0	21	0,46	0	6,5	0,22	0	15	<=0	0
30	1	5	S pep ide	SP	45,5	<=0	0	9	<=0	0	33	4 36	0	29	0,69	0	9	0,31	0	29	24,53	0
31	1	6	S pep ide	SP	23,5	<=0	0	9	<=0	0	17,5	1,81	0	20	0,43	0	8	0,27	0	19	2,11	0
32	1	7	S pep ide	SP	81	0,07	0	9	<=0	0	38	5,23	5,23	34	0,84	0	12	0,42	0	50	65,59	65,59
33	1	8	S pep ide	SP	78	0,04	0	10	<=0	0	32	4,18	0	41	1,05	0	12	0,42	0	33	32,69	0
34	2	1	S pep ide	SP	302	3,98	0	15	<=0	0	243	49,41	49,41	151	4,63	4,63	90	3,06	3,06	436,5	706,91	706,91
35	2	2	S pep ide	SP	308,5	4,12	0	33	0,59	0	172	32,85	32,85	87	2,50	0	89,5	3,05	3,05	508	822,51	822,51
36	2	3	S pep ide	SP	452	7,19	7,19	17	<=0	0	222	44,41	44,41	107	3,15	3,15	75	2,57	2,57	375,5	608,48	608,48
37	2	4	S pep ide	SP	267,5	3,30	0	10,5	<=0	0	112	19,73	19,73	56	1,51	0	26	0,92	0	170	273,80	273,8
38	2	5	S pep ide	SP	446,5	7,06	7,06	26	0,31	0	303	64,13	64,13	165	5,12	5,12	152,5	5,08	5,08	674,5	1094,22	1094,22
39	2	6	S pep ide	SP	723	13,65	13,65	44	1,05	0	397	88 29	88,29	182	5,71	5,71	264	8,61	8,61	911,5	1491,08	1491,08
40	2	7	S pep ide	SP	290,5	3,75	0	17	<=0	0	176	33,75	33,75	77	2,17	0	59	2,04	0	198,5	320,97	320,97
41	2	8	S pep ide	SP	269	3,33	0	13	<=0	0	178,5	34,32	34,32	144	4,40	4,40	87	2,96	2,96	350	567,33	567,33
42	3	1	S pep ide	SP	553	9,50	9,50	34	0,63	0	1090,5	298,53	298,53	836	32,17	32,17	850	27,38	27,38	1542	2640,12	2640,12
43	3	2	S pep ide	SP	297,5	3,89	0	22	0,16	0	184	35,57	35,57	230	7,41	7,41	497,5	15,98	15,98	1248	2084,32	2084,32
44	3	3	S pep ide	SP	1721	43,68	43,68	33	0,59	0	869	225,90	225,90	383	13,13	13,13	1383	45,75	45,75	1735,5	3029,31	3029,31
45	3	4	S pep ide	SP	1594,5	39,34	39,34	55	1,52	0	845	218,32	218,32	217,5	6,97	6,97	461,5	14,84	14,84	936	1532,98	1532,98
46	3	5	S pep ide	SP	921	18,85	18,85	17	<=0	0	552,5	130,83	130,83	224	7,20	7,20	613	19,67	19,67	1182	1964,71	1964,71
47	3	6	S pep ide	SP	429,5	6,69	6,69	17	<=0	0	285,5	59,77	59,77	182	5,71	5,71	419	13,50	13,50	864	1410,36	1410,36
48	3	7	S pep ide	SP	382,5	5,66	5,66	17	<=0	0	207,5	41,01	41,01	183,5	5,76	5,76	615,5	19,75	19,75	1009	1658,93	1658,93
49	3	8	S pep ide	SP	605	10,74	10,74	20	0,08	0	375	82 52	82,52	205,5	6,54	6,54	483	15,52	15,52	831	1354,65	1354,65
52	1	6	PMA Iono	SP	5638	283,37	283,37	4671,5	414,22	414,22	1682	515,81	515,81	4643	470,16	470,16	6211	441,29	441,29	1531,5	2619,57	2619,57
51	1	7	PMA Iono	SP	4136	161,80	161,80	5052	470,40	470,40	1556	466,69	466,69	4764	518,54	518,54	6473	501,59	501,59	1571,5	2698,16	2698,16
50	1	8	PMA Iono	SP	4102	159,57	159,57	7938,5	1092,17	1092,17	1234 5	348 31	348,31	4334,5	378,81	378,81	5295	296,02	296,02	1397,5	2362,01	2362,01
60	2	5	PMA Iono	SP	1610	39,87	39,87	6150,5	661,29	661,29	2882	1065,19	1065,19	4961	626,92	626,92	4831	245,44	245,44	1761	3082,15	3082,15
59	2	6	PMA Iono	SP	3768,5	138,70	138,70	7970	1101,46	1101,46	4470	2065,48	2065,48	5630,5	1732,84	731,2	5489	320,78	320,78	1775	3111,32	3111,32
58	2	7	PMA Iono	SP	1620,5	40,22	40,22	3044,5	219,53	219,53	2258	760,46	760,46	5527	1732,84	731,2	4976,5	260,16	260,16	1813	3191,10	3191,1
66	3	1	PMA Iono	SP	1616,5	40,09	40,09	5670	572,11	572,11	1719	530,52	530,52	4811,5	540,57	540,57	5771,5	361,79	361,79	1645	2844,76	2844,76
67	3	4	PMA Iono	SP	2902,5	92,23	92,23	4899	447,25	447,25	3504,5	1415,19	1415,19	5162	832,46	731,2	6452	496,30	496,30	1568	2691,25	2691,25
68	3	7	PMA Iono	SP	1295	29,70	29,70	4521	393,25	393,25	2322	789,75	789,75	5321	1977,29	731,2	6617	540,43	540,43	1864	3299,55	3299,55

Table 25: Cytokine raw data and calculated data for mCorVAC#15, part 5 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ LN, lymph node. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
53	2	1	Medium	LN	39,5	0,31	0	10	<=0	0	22	<=0		6	<=0	0	269	5,13	5,13
54	2	2	Medium	LN	44	0,33	0	10	<=0	0	22	<=0		7	0,08	0	432,5	8,34	8,34
55	2	3	Medium	LN	28	0,25	0	9	<=0	0	14	<=0		6	<=0	0	234,5	4,45	4,45
56	3	1	Medium	LN	62,5	0,43	0	9	<=0	0	16	<=0		6	<=0	0	355	6,81	6,81
57	3	3	Medium	LN	235	1,63	1,63	9	<=0	0	20	<=0		7	0,08	0	166	3,12	3,12
61	3	4	Medium	LN	68	0,47	0	9	<=0	0	14	<=0		8	0,1	0	204	3,86	3,86
62	3	5	Medium	LN	457	3,68	3,68	12	<=0	0	18	<=0		7	0,08	0	786	15,48	15,48
63	3	7	Medium	LN	57	0,40	0	9	<=0	0	17	<=0		7	0,08	0	157,5	2,95	2,95
64	3	8	Medium	LN	99	0,65	0	8,5	<=0	0	19	<=0		6	<=0	0	782	15,39	15,39
69	2	1	S peptide	LN	561,5	4,81	4,81	11	<=0	0	33	0,26	0	6	<=0	0	208,5	3,95	3,95
70	2	2	S peptide	LN	1013	10,70	10,70	14	<=0	0	118	3,88	3,88	7	0,08	0	1873,5	40,01	40,01
71	2	3	S peptide	LN	938,5	9,61	9,61	12	<=0	0	53	1,07	0	7	0,08	0	436	8,41	8,41
72	3	1	S peptide	LN	678	6,17	6,17	12	<=0	0	31	0,18	0	8	0,1	0	703	13,77	13,77
73	3	3	S peptide	LN	916	9,30	9,30	11,5	<=0	0	40	0,54	0	7	0,08	0	331	6,34	6,34
77	3	4	S peptide	LN	1924	27,51	27,51	14	<=0	0	43	0,66	0	7	0,08	0	723,5	14,19	14,19
78	3	5	S peptide	LN	1095	11,94	11,94	13,5	<=0	0	34,5	0,32	0	7	0,08	0	367	7,04	7,04
79	3	7	S peptide	LN	1686	22,46	22,46	13	<=0	0	51	0,98	0	6	<=0	0	701	13,73	13,73
80	3	8	S peptide	LN	564	4,83	4,83	10	<=0	0	19,5	<=0	0	6	<=0	0	658,5	12,86	12,86
74	2	1	PMA Iono	LN	4496,5	121,48	121,48	294	3,17	3,17	6532	662,77	662,77	59	1,24	1,24	17418	5,85E+07	5250
75	2	3	PMA Iono	LN	4808,5	139,72	139,72	406	4,88	4,88	8767	1352,13	1352,13	66	1,41	1,41	19327	5,85E+07	5250
76	2	7	PMA Iono	LN	3138,5	61,57	61,57	258	2,63	2,63	8778	1357,23	1357,23	51	1,04	1,04	16182	3,09E+05	5250
81	3	3	PMA Iono	LN	2999	56,86	56,86	232	2,25	2,25	6309,5	618,58	618,58	51	1,04	1,04	16058,5	1,54E+05	5250
65	3	4	PMA Iono	LN	3287	66,84	66,84	248	2,49	2,49	8188,5	1114,99	1114,99	57	1,19	1,19	17501	5,85E+07	5250
1	3	7	PMA Iono	LN	3505	75,08	75,08	281	2,98	2,98	7593,5	921,89	921,89	66,5	1,42	1,42	16859,50	5,85E+07	5250

Table 26: Cytokine raw data and calculated data for mCorVAC#15, part 6 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
53	2	1	Medium	LN	9	<=0	0	25	0,27	0	12	0,99	0	28	0,66	0	9	0,31	0	15	<=0	0
54	2	2	Medium	LN	7	<=0	0	32,5	0,57	0	15	1,43	0	21	0,46	0	7	0,24	0	14	<=0	0
55	2	3	Medium	LN	6,5	<=0	0	19	0,05	0	11	0,85	0	21	0,46	0	7	0,24	0	13	<=0	0
56	3	1	Medium	LN	7	<=0	0	10	<=0	0	12	0,99	0	22	0,49	0	7	0,24	0	15	<=0	0
57	3	3	Medium	LN	10	<=0	0	20	0,08	0	13	1,13	0	29	0,69	0	10	0,35	0	26	18,22	0
61	3	4	Medium	LN	9	<=0	0	13	<=0	0	14	1,28	0	33	0,81	0	6	<=0	0	14	<=0	0
62	3	5	Medium	LN	15	<=0	0	10	<=0	0	12	0,99	0	26	0,60	0	8	0,27	0	41,5	49,42	0
63	3	7	Medium	LN	13	<=0	0	14	<=0	0	15	1,43	0	29	0,69	0	6	<=0	0	14,5	<=0	0
64	3	8	Medium	LN	12	<=0	0	15	<=0	0	15	1,43	0	26	0,60	0	7	0,24	0	17	<=0	0
69	2	1	S pep ide	LN	12,5	<=0	0	30	0,47	0	13	1,13	0	34	0,84	0	11	0,39	0	52,5	70,27	70,27
70	2	2	S pep ide	LN	46,5	<=0	0	215	9,08	9,08	23	2,68	0	46,5	1,22	0	47	1,64	0	83,5	126,35	126,35
71	2	3	S pep ide	LN	11	<=0	0	96,5	3,38	3,38	15	1,43	0	33	0,81	0	20	0,71	0	82	123,69	123,69
72	3	1	S pep ide	LN	32	<=0	0	20	0,08	0	14	1,28	0	31	0,75	0	17	0,60	0	66,5	95,97	95,97
73	3	3	S pep ide	LN	25	<=0	0	16	<=0	0	13	1,13	0	30,5	0,74	0	17,5	0,62	0	76	113,04	113,04
77	3	4	S pep ide	LN	44,5	<=0	0	15	<=0	0	21	2 36	0	54	1,45	0	31	1,09	0	156	250,44	250,44
78	3	5	S pep ide	LN	54	<=0	0	18	0,01	0	15	1,43	0	40	1,02	0	21	0,75	0	91	139,53	139,53
79	3	7	S pep ide	LN	49,5	<=0	0	27,5	0,37	0	18,5	1,96	0	45	1,17	0	26	0,92	0	136,5	217,66	217,66
80	3	8	S pep ide	LN	20	<=0	0	14	<=0	0	15	1,43	0	26	0,60	0	11	0,39	0	52	69,33	69,33
74	2	1	PMA Iono	LN	1662	41,64	41,64	8746	1353,47	1353,47	359,5	78,50	78,50	5696	1732,84	731,2	3332,5	133,64	133,64	1848	3265,35	3265,35
75	2	3	PMA Iono	LN	1720	43,64	43,64	11982	3124,01	3124,01	383	84,61	84,61	6322,5	1732,84	731,2	3394	137,20	137,20	1879,5	3332,84	3332,84
76	2	7	PMA Iono	LN	1056,5	22,63	22,63	10201	1974,51	1974,51	569	135,52	135,52	5226	962,86	731,2	1981	68,61	68,61	1547	2649,93	2649,93
81	3	3	PMA Iono	LN	737	14,00	14,00	7712	1027,35	1027,35	257,5	52,91	52,91	4908	592,68	592,68	3682	154,80	154,80	1503	2564,06	2564,06
65	3	4	PMA Iono	LN	853	17,02	17,02	7043,5	854,66	854,66	435	98,41	98,41	5309	1469,86	731,2	5271,5	293,18	293,18	1475,5	2510,88	2510,88
1	3	7	PMA Iono	LN	943,5	19,47	19,47	7862	1069,89	1069,89	491	113,65	113,65	5508,5	1732,84	731,2	4949	257,31	257,31	1666	2887,17	2887,17

Table 27: Cytokine raw data and calculated data for mCorVAC#16, part 1 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	1	1	Medium	SP	98,5	2,06	2,06	13	<=0	0	35	2,39	2,39	23	1,64	1,64	971,5	59,72	59,72
1	2	1	2	Medium	SP	20	0,17	0	10	<=0	0	24	0,70	0	17	0,94	0	106	9,49	9,49
1	3	1	3	Medium	SP	76,5	1,50	1,50	13	<=0	0	41	3,25	3,25	16	0,82	0	646,5	42,56	42,56
1	4	1	4	Medium	SP	78	1,54	1,54	11	<=0	0	29	1,49	0	22	1,53	1,53	692,5	45,05	45,05
1	5	1	5	Medium	SP	26	0,30	0	9	<=0	0	23	0,53	0	18	1,06	1,06	404,5	28,98	28,98
1	6	1	6	Medium	SP	236	5,80	5,80	13	<=0	0	42	3,40	3,40	18	1,06	1,06	354	25,99	25,99
1	7	1	7	Medium	SP	259,5	6,47	6,47	13	<=0	0	28	1,34	0	18,5	1,12	1,12	426	30,24	30,24
1	8	1	8	Medium	SP	53	0,92	0	15	<=0	0	23	0,53	0	20	1,30	1,30	776	49,50	49,50
1	9	2	1	Medium	SP	44	0,71	0	10	<=0	0	24	0,70	0	15	0,69	0	245	19,22	19,22
1	10	2	2	Medium	SP	275	6,91	6,91	17	<=0	0	59	5,72	5,72	16	0,82	0	955	58,87	58,87
1	11	2	3	Medium	SP	161	3,71	3,71	26,5	0,5	0	95	10,38	10,38	19	1,18	1,18	848	53,29	53,29
1	12	2	4	Medium	SP	228	5,57	5,57	17	<=0	0	52	4,78	4,78	16	0,82	0	773	49,34	49,34
1	13	2	5	Medium	SP	1728	57,82	57,82	27	0,53	0	93	10,12	10,12	18	1,06	1,06	1119,5	67,33	67,33
1	14	2	6	Medium	SP	54	0,95	0	12	<=0	0	22	0,35	0	17	0,94	0	226,5	18,02	18,02
1	15	2	7	Medium	SP	128,5	2,84	2,84	13	<=0	0	47	4,09	4,09	15	0,69	0	953,5	58,79	58,79
1	16	2	8	Medium	SP	2259,5	80,30	80,30	27,5	0,55	0	68	6,91	6,91	20	1,30	1,30	1375,5	80,35	80,35
1	17	3	1	Medium	SP	79	1,56	1,56	10	<=0	0	27	1,18	0	18	1,06	1,06	132	11,47	11,47
1	18	3	2	Medium	SP	79	1,56	1,56	12	<=0	0	29	1,49	0	19	1,18	1,18	296	22,45	22,45
1	19	3	3	Medium	SP	84,5	1,70	1,70	11	<=0	0	25	0,86	0	23	1,64	1,64	142,5	12,24	12,24
1	20	3	4	Medium	SP	919,5	27,52	27,52	22	0,27	0	80	8,47	8,47	22	1,53	1,53	956,5	58,95	58,95
1	21	3	5	Medium	SP	322,5	8,30	8,30	16	<=0	0	60	5,86	5,86	33	2,72	2,72	869	54,39	54,39
1	22	3	6	Medium	SP	130	2,88	2,88	12,5	<=0	0	44	3,68	3,68	19,5	1,24	1,24	374	27,18	27,18
1	23	3	7	Medium	SP	108,5	2,32	2,32	12	<=0	0	32,5	2,02	0	22	1,53	1,53	189,5	15,54	15,54
1	24	3	8	Medium	SP	168	3,90	3,90	13	<=0	0	39,5	3,04	3,04	17	0,94	0	1218	72,35	72,35

Table 28: Cytokine raw data and calculated data for mCorVAC#16, part 2 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	1	Medium	SP	106,5	5,5	5,5	10	<=0	0	48	26,82	26,82	49	5,51	5,51	7	<=0	0	21	<=0	0
2	1	2	Medium	SP	21,5	0,8	0	11	<=0	0	18	5,99	5,99	26	2,49	0	5	<=0	0	13	<=0	0
3	1	3	Medium	SP	91	4,7	4,7	12	<=0	0	43,5	23,94	23,94	34	3,61	3,61	8	<=0	0	17	<=0	0
4	1	4	Medium	SP	83	4,2	4,2	11	<=0	0	43	23,61	23,61	44	4,90	4,90	8	<=0	0	17	<=0	0
5	1	5	Medium	SP	23	0,9	0	11	<=0	0	29	14,20	14,20	26,5	2,57	0	5,5	<=0	0	14	<=0	0
6	1	6	Medium	SP	429	22,0	22,0	19	0,2	0	129,5	73,96	73,96	41	4,52	4,52	7	<=0	0	29	114,63	0
7	1	7	Medium	SP	63	3,2	3,2	10	<=0	0	30	14,90	14,90	38	4,14	4,14	8	<=0	0	32	144,62	0
8	1	8	Medium	SP	50	2,4	2,4	9	<=0	0	185	103,35	103,35	652,5	56,48	56,48	7	<=0	0	16,5	<=0	0
9	2	1	Medium	SP	86,5	4,4	4,4	12	<=0	0	34	17,65	17,65	32	3,34	3,34	7	<=0	0	13	<=0	0
10	2	2	Medium	SP	487,5	24,9	24,9	12	<=0	0	122	69,87	69,87	77	8,68	8,68	19	4,13	0	38,5	202,86	202,86
11	2	3	Medium	SP	1087,5	55,5	55,5	12	<=0	0	297	159,65	159,65	69	7,81	7,81	14	2,36	0	29	114,63	0
12	2	4	Medium	SP	458	23,4	23,4	28	2,39	0	128	73,14	73,14	57	6,45	6,45	8	<=0	0	27	92,89	0
13	2	5	Medium	SP	1096	55,9	55,9	15	<=0	0	292,5	157,45	157,45	67	7,59	7,59	19,5	4,29	0	162	929,78	929,78
14	2	6	Medium	SP	206,5	10,7	10,7	14	<=0	0	60,5	34,59	34,59	34	3,61	3,61	6	<=0	0	14	<=0	0
15	2	7	Medium	SP	294,5	15,2	15,2	9	<=0	0	105	60,46	60,46	51,5	5,81	5,81	11	0,87	0	21,5	14,31	0
16	2	8	Medium	SP	395	20,3	20,3	12	<=0	0	163	91,86	91,86	529	47,07	47,07	17	3,48	0	198	1098,85	1098,85
17	3	1	Medium	SP	69,5	3,5	3,5	10	<=0	0	33	16,97	16,97	32	3,34	3,34	7	<=0	0	16	<=0	0
18	3	2	Medium	SP	114	5,9	5,9	13	<=0	0	39	21,00	21,00	37	4,01	4,01	7	<=0	0	17	<=0	0
19	3	3	Medium	SP	63	3,2	3,2	12	<=0	0	34,5	17,99	17,99	37	4,01	4,01	8	<=0	0	18	<=0	0
20	3	4	Medium	SP	608,5	31,0	31,0	12	<=0	0	174	97,63	97,63	64	7,25	7,25	18	3,81	0	78	479,51	479,51
21	3	5	Medium	SP	345	17,7	17,7	14	<=0	0	122	69,87	69,87	51	5,75	5,75	10	<=0	0	34	163,35	0
22	3	6	Medium	SP	219	11,3	11,3	9	<=0	0	92	53,11	53,11	36	3,88	3,88	8	<=0	0	21	<=0	0
23	3	7	Medium	SP	211,5	11,0	11,0	17	<=0	0	69,5	40,01	40,01	41	4,52	4,52	9	<=0	0	20	<=0	0
24	3	8	Medium	SP	144	7,5	7,5	14	<=0	0	47	26,18	26,18	41	4,52	4,52	8	<=0	0	23,5	48,81	0

Table 29: Cytokine raw data and calculated data for mCorVAC#16, part 3 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	25	1	1	S peptide	SP	60,5	1,10	1,10	10	<=0	0	24	0,70	0	19	1,18	1,18	346	25,51	25,51
1	26	1	2	S peptide	SP	70	1,34	1,34	10	<=0	0	24	0,70	0	16	0,82	0	101	9,10	9,10
1	27	1	3	S peptide	SP	53	0,92	0	9,5	<=0	0	21	0,17	0	14	0,56	0	122,5	10,76	10,76
1	28	1	4	S peptide	SP	182,5	4,30	4,30	12	<=0	0	27	1,18	0	20,5	1,36	1,36	348	25,63	25,63
1	29	1	5	S peptide	SP	46	0,75	0	14	<=0	0	31	1,80	0	15,5	0,76	0	151	12,85	12,85
1	30	1	6	S peptide	SP	781,5	22,82	22,82	17,5	0,03	0	66,5	6,72	6,72	19	1,18	1,18	697	45,29	45,29
1	31	1	7	S peptide	SP	276	6,94	6,94	12	<=0	0	23	0,53	0	18	1,06	1,06	331	24,60	24,60
1	32	1	8	S peptide	SP	248,5	6,15	6,15	14	<=0	0	29	1,49	0	25	1,87	1,87	351	25,81	25,81
1	33	2	1	S peptide	SP	13171	5808,01	4800	174	7,19	7,19	3368	382,28	382,28	48	4,21	4,21	1871	105,49	105,49
1	34	2	2	S peptide	SP	14077	5808,01	4800	184,5	7,65	7,65	5418	676,64	676,64	65	5,80	5,80	3257	179,56	179,56
1	35	2	3	S peptide	SP	15533	5808,01	4800	210	8,78	8,78	4642	555,60	555,60	97	8,64	8,64	1595	91,47	91,47
1	36	2	4	S peptide	SP	13978	5808,01	4800	180	7,45	7,45	4989,5	608,07	608,07	58	5,16	5,16	2848	156,78	156,78
1	37	2	5	S peptide	SP	13461	5808,01	4800	164	6,75	6,75	3984,5	462,82	462,82	52	4,59	4,59	2266,5	125,84	125,84
1	38	2	6	S peptide	SP	13336,5	5808,01	4800	169	6,97	6,97	4404	521,10	521,10	61	5,44	5,44	2555	141,01	141,01
1	39	2	7	S peptide	SP	12383	5808,01	4800	170	7,01	7,01	5234	646,64	646,64	52	4,59	4,59	2893,5	159,27	159,27
1	40	2	8	S peptide	SP	17133	5808,01	4800	236	9,93	9,93	4725	567,90	567,90	89,5	7,99	7,99	1762	99,94	99,94
1	41	3	1	S peptide	SP	13734	5808,01	4800	121,5	4,86	4,86	505	57,29	57,29	58	5,16	5,16	1366	79,87	79,87
1	42	3	2	S peptide	SP	13464	5808,01	4800	119	4,75	4,75	653	73,40	73,40	54	4,78	4,78	1191	70,98	70,98
1	43	3	3	S peptide	SP	13090,5	5808,01	4800	112	4,44	4,44	610	68,74	68,74	59	5,25	5,25	954	58,82	58,82
1	44	3	4	S peptide	SP	15944	5808,01	4800	159	6,53	6,53	1533	168,50	168,50	76	6,80	6,80	2185	121,61	121,61
1	45	3	5	S peptide	SP	14532,5	5808,01	4800	152	6,22	6,22	2293,5	253,39	253,39	87	7,77	7,77	1549	89,14	89,14
1	46	3	6	S peptide	SP	15439	5808,01	4800	151	6,17	6,17	891	99,08	99,08	61	5,44	5,44	2981	164,08	164,08
1	47	3	7	S peptide	SP	13213,5	5808,01	4800	124	4,98	4,98	1358	149,45	149,45	58	5,16	5,16	1839,5	103,88	103,88
1	48	3	8	S peptide	SP	13278	5808,01	4800	104	4,08	4,08	414	47,28	47,28	53	4,69	4,69	1581	90,76	90,76
1	49	1	1	PMA Iono	SP	4486	201,33	201,33	350	15,01	15,01	9735	1951,80	1951,80	97	8,64	8,64	16018	5,7E+04	1312,5
1	50	1	2	PMA Iono	SP	4369,5	193,67	193,67	399,5	17,25	17,25	11225	4223,94	2162,5	108	9,58	9,58	15895	5,7E+04	1312,5
1	51	1	3	PMA Iono	SP	4254	186,26	186,26	389	16,77	16,77	10268	2346,54	2162,5	104	9,24	9,24	15356	5,7E+04	1312,5
1	52	2	1	PMA Iono	SP	4815	223,94	223,94	348	14,92	14,92	11244	4327,85	2162,5	134,5	11,80	11,80	16167	5,7E+04	1312,5
1	53	2	2	PMA Iono	SP	6032	323,14	323,14	271	11,48	11,48	11185	4031,79	2162,5	99	8,81	8,81	15750,5	5,7E+04	1312,5
1	54	2	3	PMA Iono	SP	5693	292,66	292,66	281,5	11,94	11,94	10321	2395,89	2162,5	111	9,84	9,84	13797	5,7E+04	1312,5
1	55	3	1	PMA Iono	SP	4782,5	221,64	221,64	403,5	17,43	17,43	11140	3848,56	2162,5	120	10,60	10,60	16203,5	5,7E+04	1312,5
1	56	3	2	PMA Iono	SP	5422	270,00	270,00	323	13,80	13,8	10867	3124,27	2162,5	103	9,16	9,16	15723	5,7E+04	1312,5
1	57	3	3	PMA Iono	SP	5531	278,95	278,95	261,5	11,06	11,06	9846	2022,03	2022,03	98	8,73	8,73	14422	5,7E+04	1312,5

Table 30: Cytokine raw data and calculated data for mCorVAC#16, part 4 of 6 (SP)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control). SP, spleen.

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
25	1	1	S pep ide	SP	47	2,3	2,3	9	<=0	0	41	22,31	22,31	52	5,87	5,87	7	<=0	0	15	<=0	0
26	1	2	S pep ide	SP	68	3,4	3,4	11,5	<=0	0	32	16,29	16,29	32	3,34	3,34	8	<=0	0	16	<=0	0
27	1	3	S pep ide	SP	58	2,9	2,9	10	<=0	0	37	19,67	19,67	28,5	2,86	2,86	6	<=0	0	14	<=0	0
28	1	4	S pep ide	SP	121	6,3	6,3	9	<=0	0	65,5	37,61	37,61	44	4,90	4,90	8	<=0	0	25	69,01	0
29	1	5	S pep ide	SP	320	16,5	16,5	9	<=0	0	89,5	51,68	51,68	33	3,48	3,48	7	<=0	0	16	<=0	0
30	1	6	S pep ide	SP	545	27,8	27,8	14	<=0	0	183	102,32	102,32	53	5,99	5,99	15	2,76	0	71	435,82	435,82
31	1	7	S pep ide	SP	167	8,7	8,7	10	<=0	0	50	28,08	28,08	38	4,14	4,14	7	<=0	0	30	124,92	0
32	1	8	S pep ide	SP	171	8,9	8,9	9,5	<=0	0	69	39,71	39,71	245,5	24,38	24,38	10	<=0	0	35	172,42	0
33	2	1	S pep ide	SP	3097	175,0	175,0	80,5	11,50	11,5	1939	921,39	921,39	748,5	63,72	63,72	1357	133,31	133,31	1730	6283,48	6283,48
34	2	2	S pep ide	SP	2500	135,8	135,8	178,5	25,53	25,53	1790	850,48	850,48	719	61,50	61,50	1401	136,55	136,55	2007,5	7154,03	7154,03
35	2	3	S pep ide	SP	2530	137,7	137,7	120,5	17,47	17,47	2662	1280,76	1280,76	882	73,74	73,74	2013	180,57	180,57	2382	8351,97	8351,97
36	2	4	S pep ide	SP	2212,5	118,2	118,2	321	43,79	43,79	1915,5	910,15	910,15	694	59,61	59,61	1559	148,08	148,08	1846,5	6647,80	6647,80
37	2	5	S pep ide	SP	2365,5	127,5	127,5	271	37,56	37,56	2038	969,03	969,03	601,5	52,61	52,61	840	93,57	93,57	1806	6521,00	6521,00
38	2	6	S pep ide	SP	1640	85,2	85,2	146,5	21,15	21,15	1583,5	753,58	753,58	830	69,84	69,84	1562,5	148,33	148,33	1889,5	6782,64	6782,64
39	2	7	S pep ide	SP	2880	160,3	160,3	200	28,40	28,4	2046	972,90	972,90	827	69,61	69,61	1447	139,92	139,92	1678,5	6122,76	6122,76
40	2	8	S pep ide	SP	1653	85,9	85,9	87,5	12,58	12,58	2760,5	1332,05	1332,05	2128,5	173,56	173,56	2153,5	190,53	190,53	2511	8773,84	8773,84
41	3	1	S pep ide	SP	919	46,8	46,8	24	1,52	0	620	312,22	312,22	657	56,82	56,82	460	60,65	60,65	1982	7073,58	7073,58
42	3	2	S pep ide	SP	839,5	42,7	42,7	29	2,60	0	488,5	251,16	251,16	680	58,56	58,56	509	65,21	65,21	1791	6474,08	6474,08
43	3	3	S pep ide	SP	477	24,4	24,4	133	19,26	19,26	334	177,69	177,69	551,5	48,80	48,80	368	51,71	51,71	1829,5	6594,56	6594,56
44	3	4	S pep ide	SP	787,5	40,0	40,0	40	4,74	0	786	388,32	388,32	1065	87,50	87,50	1142	117,21	117,21	2457,5	8598,20	8598,20
45	3	5	S pep ide	SP	1429	73,6	73,6	71	10,01	10,01	1561	743,11	743,11	803	67,81	67,81	860	95,18	95,18	2058	7313,69	7313,69
46	3	6	S pep ide	SP	668,5	34,0	34,0	60	8,22	8,22	624	314,07	314,07	1027	84,63	84,63	954	102,67	102,67	2260	7957,81	7957,81
47	3	7	S pep ide	SP	825	42,0	42,0	78	11,11	11,11	494	253,73	253,73	587	51,51	51,51	656	78,24	78,24	1859,5	6688,55	6688,55
48	3	8	S pep ide	SP	454	23,2	23,2	34	3,60	0	294	158,18	158,18	631,5	54,89	54,89	393	54,19	54,19	1951	6975,93	6975,93
49	1	1	PMA Iono	SP	2056	108,9	108,9	6519,5	872,65	872,65	3366	1662,56	1662,56	5721	4142,40	731,2	5495	461,34	461,34	1671	6099,37	6099,37
50	1	2	PMA Iono	SP	3480	202,3	202,3	9358,5	1609,56	1609,56	4770	2563,19	2563,19	5510,5	1256,15	731,2	5864	502,13	502,13	1411	5288,61	5288,61
51	1	3	PMA Iono	SP	1521	78,6	78,6	7790,5	1147,31	1147,31	4659	2483,32	2483,32	5649,5	1891,68	731,2	5892,5	505,47	505,47	1638	5996,47	5996,47
52	2	1	PMA Iono	SP	1759	91,8	91,8	4315,5	516,09	516,09	4106	2109,67	2109,67	5675	2216,16	731,2	6114	532,52	532,52	1684	6139,92	6139,92
53	2	2	PMA Iono	SP	1338	68,7	68,7	5758	735,82	735,82	3354	1655,73	1655,73	5067	752,98	731,2	6086	528,98	528,98	1184	4576,36	4576,36
54	2	3	PMA Iono	SP	1183	60,5	60,5	5530,5	698,08	698,08	3337	1646,08	1646,08	4612	557,74	557,74	5094	421,22	421,22	1063	4192,38	4192,38
55	3	1	PMA Iono	SP	1795,5	93,9	93,9	10589	2139,77	2000	4271	2217,20	2217,20	6012	4142,40	731,2	5849	500,39	500,39	1660,5	6066,62	6066,62
56	3	2	PMA Iono	SP	870	44,2	44,2	11204,5	2499,94	2000	3363,5	1661,14	1661,14	5047	741,29	731,2	5963	513,87	513,87	1215,5	4675,72	4675,72
57	3	3	PMA Iono	SP	1056,5	53,9	53,9	8771	1415,41	1415,41	2883	1396,74	1396,74	4732	598,42	598,42	4594	375,76	375,76	1068	4208,33	4208,33

Table 31: Cytokine raw data and calculated data for mCorVAC#16, part 5 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

						IFN-gamma			IL-12p70			IL-13			IL-1beta			IL-2		
Plate	Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
2	1	1	2	Medium	LN	30	0,06	0	10	<=0	0	32,5	2,58	2,58	6	0,27	0	172	14,15	14,15
2	2	1	3	Medium	LN	35,5	0,23	0	9	<=0	0	32	2,52	2,52	6	0,27	0	520	33,53	33,53
2	3	1	5	Medium	LN	32	0,12	0	10	<=0	0	29	2,18	2,18	7	0,40	0	51	5,29	5,29
2	7	2	1	Medium	LN	93	1,76	1,76	9	<=0	0	31	2,41	2,41	6	0,27	0	190	15,30	15,30
2	8	2	2	Medium	LN	159	3,37	3,37	10	<=0	0	24	1,60	0	7	0,40	0	275,5	20,43	20,43
2	9	2	3	Medium	LN	194	4,20	4,20	8	<=0	0	24	1,60	0	8	0,52	0	130	11,36	11,36
2	10	2	4	Medium	LN	427	9,61	9,61	11	<=0	0	33	2,63	2,63	7	0,40	0	247	18,76	18,76
2	11	2	5	Medium	LN	86	1,58	1,58	11	<=0	0	35	2,85	2,85	7	0,40	0	503	32,66	32,66
2	12	2	6	Medium	LN	106	2,08	2,08	10	<=0	0	63,5	5,86	5,86	7	0,40	0	693,5	42,09	42,09
2	13	2	7	Medium	LN	452,5	10,20	10,20	11	<=0	0	85	8,01	8,01	8	0,52	0	373	25,86	25,86
2	14	2	8	Medium	LN	275	6,10	6,10	11	<=0	0	43	3,73	3,73	7	0,40	0	407	27,68	27,68
2	4	3	1	Medium	LN	88	1,63	1,63	10	<=0	0	27	1,95	0	7	0,40	0	247	18,76	18,76
2	5	3	2	Medium	LN	118	2,38	2,38	9	<=0	0	27	1,95	0	6	0,27	0	227	17,57	17,57
2	6	3	5	Medium	LN	3862,5	112,04	112,04	28	0,72	0	65,5	6,06	6,06	21	1,83	1,83	722	43,46	43,46
2	15	1	2	S peptide	LN	27	<=0	0	9	<=0	0	30,5	2,35	2,35	6	0,27	0	144	12,31	12,31
2	16	1	3	S peptide	LN	24	<=0	0	9	<=0	0	24,5	1,66	0	6	0,27	0	42,5	4,52	4,52
2	17	1	5	S peptide	LN	38	0,30	0	9	<=0	0	23	1,48	0	6	0,27	0	39	4,19	4,19
2	21	2	1	S peptide	LN	6019	228,22	228,22	43	1,39	0	1158	98,84	98,84	13	1,06	1,06	3419	169,20	169,20
2	22	2	2	S peptide	LN	9299	625,33	625,33	71	2,57	2,57	1738	147,12	147,12	17	1,45	1,45	3274	161,90	161,90
2	23	2	3	S peptide	LN	12071	1686,02	1686,02	80	2,94	2,94	1253	106,68	106,68	25,5	2,23	2,23	2581	128,41	128,41
2	24	2	4	S peptide	LN	6145	237,22	237,22	45	1,47	0	1326	112,68	112,68	13	1,06	1,06	3027	149,72	149,72
2	25	2	5	S peptide	LN	5060	168,77	168,77	50	1,69	1,69	2139	181,51	181,51	12	0,96	0	3253	160,86	160,86
2	26	2	6	S peptide	LN	3496	97,61	97,61	28,5	0,74	0	672	58,84	58,84	10	0,74	0	2548	126,87	126,87
2	27	2	7	S peptide	LN	4729	151,42	151,42	42	1,34	0	3101	268,62	268,62	14	1,16	1,16	2190	110,32	110,32
2	28	2	8	S peptide	LN	7910	405,40	405,40	54	1,86	1,86	1622	137,39	137,39	19	1,64	1,64	2899,5	143,55	143,55
2	18	3	1	S peptide	LN	1803,5	43,49	43,49	13	<=0	0	41	3,51	3,51	7	0,40	0	701,5	42,47	42,47
2	19	3	2	S peptide	LN	3364,5	92,70	92,70	20	0,34	0	87	8,20	8,20	8	0,52	0	553,5	35,21	35,21
2	20	3	5	S peptide	LN	4311	131,42	131,42	27	0,67	0	138	13,06	13,06	33	2,87	2,87	1182,5	64,83	64,83
2	29	1	1	PMA Iono	LN	12326	1874,34	1874,34	196	7,52	7,52	5467	527,14	527,14	37	3,20	3,20	16127	8492,61	5250
2	30	2	1	PMA Iono	LN	12488	2008,41	2008,41	333	12,79	12,79	8110	953,50	953,50	65	5,36	5,36	18009	5,2E+05	5250
2	31	2	2	PMA Iono	LN	13892	3937,75	3937,75	232	8,91	8,91	7472	830,47	830,47	57,5	4,80	4,80	17831	1,5E+05	5250
2	32	3	1	PMA Iono	LN	13092,5	2635,94	2635,94	287	11,02	11,02	7797	891,10	891,10	65	5,36	5,36	18563	1,7E+07	5250

Table 32: Cytokine raw data and calculated data for mCorVAC#16, part 6 of 6 (LN)

Acquired MFI values (MFI) were converted into concentrations by ProcartaPlex Analysis software (c_{calc}). Final concentrations (c_{fin}) meet the following criteria: Values below the lower limit of quantification (<LLOQ, blue)) are set to zero. Values above the upper limit of quantification (>ULOQ, red) are set to the ULOQ. Commas are used as decimal separators. Thousand were not separated by commas. LN, lymph node. Gr, group. M, mouse ID. PMAIono, PMA and Ionomycin (positive control).

					IL-4			IL-5			IL-6			TNF-alpha			GM-CSF			IL-18		
Sample ID	Gr	M	Restimulation	Tissue	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]	MFI	c_{calc} [pg/mL]	c_{fin} [pg/mL]
1	1	2	Medium	LN	5	<=0	0	94	13,14	13,14	17	7,72	7,72	37,5	4,19	4,19	6	1,14	0	14	<=0	0
2	1	3	Medium	LN	6	0,23	0	67	9,43	9,43	15	6,36	6,36	32	3,57	3,57	5	<=0	0,00	13	<=0	0
3	1	5	Medium	LN	8	0,37	0	107,5	14,94	14,94	11	3,47	0	27	2,99	2,99	5	<=0	0,00	12	<=0	0
7	2	1	Medium	LN	17	0,95	0	44	6,09	6,09	15	6,36	6,36	28	3,11	3,11	7	1,50	0	18	<=0	0
8	2	2	Medium	LN	21	1,19	0	16	1,55	0	14	5,66	5,66	55	6,01	6,01	7	1,50	0	21	51,55	0
9	2	3	Medium	LN	11,5	0,61	0	12	0,77	0	17	7,72	7,72	32	3,57	3,57	6	1,14	0	22,5	70,19	0
10	2	4	Medium	LN	28	1,60	1,60	14	1,17	0	17	7,72	7,72	38	4,24	4,24	8	1,83	0	41	224,61	224,61
11	2	5	Medium	LN	33,5	1,90	1,90	39	5,33	5,33	17	7,72	7,72	47	5,20	5,20	7	1,50	0	19	19,32	0
12	2	6	Medium	LN	18,5	1,04	0	29	3,76	3,76	13	4,95	4,95	35,5	3,96	3,96	9	2,12	0	17	<=0	0,00
13	2	7	Medium	LN	23	1,31	1,31	126	17,36	17,36	16	7,04	7,04	48	5,30	5,30	16	3,78	3,78	42	231,36	231,36
14	2	8	Medium	LN	28	1,60	1,60	23	2,77	2,77	21	10,35	10,35	39	4,35	4,35	8	1,83	0	32	158,79	0
4	3	1	Medium	LN	6	0,23	0	33	4,40	4,40	11	3,47	0	25	2,74	0	5	<=0	0,00	18	<=0	0,00
5	3	2	Medium	LN	5	<=0	0,00	44	6,09	6,09	12	4,22	0	27	2,99	2,99	7	1,50	0	18	<=0	51750
6	3	5	Medium	LN	80	4,32	4,32	19	2,09	2,09	183	94,27	94,27	112	11,24	11,24	11	2,65	2,65	327	1366,33	1366,33
15	1	2	S peptide	LN	6	0,23	0	77	10,83	10,83	12	4,22	0	29	3,22	3,22	6	1,14	0	11	<=0	0,00
16	1	3	S peptide	LN	6,5	0,27	0	37	5,02	5,02	12	4,22	0	23	2,50	0	6	1,14	0	13	<=0	0,00
17	1	5	S peptide	LN	7	0,30	0	79	11,10	11,10	11	3,47	0	22,5	2,43	0	5	<=0	0,00	12	<=0	0,00
21	2	1	S peptide	LN	585	26,67	26,67	306	39,25	39,25	50,5	27,77	27,77	185	17,18	17,18	208,5	24,46	24,46	525	1945,60	1945,60
22	2	2	S peptide	LN	620	28,14	28,14	108	15,01	15,01	68,5	37,55	37,55	249	22,03	22,03	246,5	27,42	27,42	932	3012,15	3012,15
23	2	3	S peptide	LN	315	15,07	15,07	123,5	17,03	17,03	100	53,89	53,89	327,5	27,72	27,72	362	35,69	35,69	1379	4111,96	4111,96
24	2	4	S peptide	LN	722	32,50	32,50	124	17,10	17,10	51	28,04	28,04	190,5	17,61	17,61	326	33,21	33,21	566	2060,06	2060,06
25	2	5	S peptide	LN	676	30,52	30,52	838,5	98,03	98,03	41	22,40	22,40	242	21,51	21,51	333	33,69	33,69	466	1780,70	1780,70
26	2	6	S peptide	LN	374	17,63	17,63	172	23,18	23,18	35	18,92	18,92	114	11,41	11,41	176	21,79	21,79	304	1292,04	1292,04
27	2	7	S peptide	LN	388	18,23	18,23	3354,5	371,05	371,05	44	24,12	24,12	185,5	17,22	17,22	543	47,36	47,36	421	1650,50	1650,50
28	2	8	S peptide	LN	835	37,33	37,33	112	15,53	15,53	72	39,41	39,41	231,5	20,73	20,73	537	46,99	46,99	748	2542,37	2542,37
18	3	1	S peptide	LN	18	1,01	0	41	5,64	5,64	20	9,70	9,70	66	7,09	7,09	10	2,39	0	145	731,35	731,35
19	3	2	S peptide	LN	22	1,25	1,25	137	18,77	18,77	17	7,72	7,72	76	8,03	8,03	17	3,99	3,99	256	1136,68	1136,68
20	3	5	S peptide	LN	83	4,47	4,47	23	2,77	2,77	185	95,21	95,21	188	17,41	17,41	40	7,78	7,78	375	1513,77	1513,77
29	1	1	PMA Iono	LN	802	35,92	35,92	6819	837,42	837,42	951	425,96	425,96	5191,5	528,45	528,45	1086	78,50	78,50	1598	4645,82	4645,82
30	2	1	PMA Iono	LN	930	41,41	41,41	11126	1777,66	1777,66	1430	628,21	628,21	6317	924,47	731,2	4143,5	273,05	273,05	2253	6278,43	6278,43
31	2	2	PMA Iono	LN	494	22,76	22,76	7001	866,85	866,85	1107	491,32	491,32	6276	901,77	731,2	3748	241,36	241,36	2246	6260,47	6260,47
32	3	1	PMA Iono	LN	1641	73,08	73,08	6896,5	849,87	849,87	2801	1244,18	1244,18	6159,5	842,57	731,2	3033,5	190,98	190,98	2431	6740,05	6740,05

9.6 Certificates of Analysis BNT162a1

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BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBL063.3 (ATM batch uRNAv05)**
Lot/Batch No.:	RNA-SK200305-01
RNA length:	1261 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	06 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

(b) (6)

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Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBL063.3 LNP
Lot: CoVVAC/090320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 26.03.2020 (b) (6)

Date: 26.03.20 (b) (6)

(b) (6)

9.7 Certificates of Analysis BNT162b1

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Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.3 (ATM batch modRNAv05)**
Lot/Batch No.:	RNA-RF200304-03
RNA length:	1262 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	05 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

(b) (6)

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Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.3 LNP
Lot: CoVVAC/100320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 26.03.2020 (b) (6)

Date: 26.03.2020

9.8 Certificates of Analysis BNT162b2

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Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.2 (ATM batch modRNAv09)**
Lot/Batch No.:	RNA-RF200321-06
RNA length:	4283 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	19 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Denaturing Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

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Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.2LNP
Lot: CoVVAC/270320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 09.04.20 (b) (6)

Date: 09.04.20 (b) (6)

9.9 Certificates of Analysis BNT162c2

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BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBS004.2 (ATM batch saRNAv09)**
Lot/Batch No.:	RNA-RF200310-01
RNA length:	11917 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	09 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

(b) (6)

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Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBS004.2 LNP
Lot: CoVVAC/170320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/015)	
RNA integrity	CE (223/SOP/015)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 identification and content	HPLC-CAD (222/SOP/044)	
ALC-0159 identification and content	HPLC-CAD (222/SOP/044)	
DSPC identification and content	HPLC-CAD (222/SOP/044)	
Cholesterol identification and content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

(b) (6)

Date: 26.03.2020

Date: 26.03.2020

9.10 Statistical analysis

ELISpot – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 6

Group	Sidak's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	Summary	Adjusted P Value
Control (mCorVac#15)	No peptide vs. S pepide	-4.938	-8.321 to -1,554	Yes	**	0.0041
	Control RNA vs. S RNA	2.125	-1.258 to 5.508	No	ns	0.2696
BNT162a1	No peptide vs. S pepide	-82.81	-103.4 to -62.26	Yes	****	<0.0001
	Control RNA vs. S RNA	-47.13	-67.67 to -26.58	Yes	****	<0.0001
BNT162b1	No peptide vs. S pepide	-748.2	-894.1 to -602.3	Yes	****	<0.0001
	Control RNA vs. S RNA	-734.5	-880.4 to -588.6	Yes	****	<0.0001
Control (mCorVac#16)	No peptide vs. S peptide	-14.50	-22.73 to -6.270	Yes	***	0.0007
	Control RNA vs. S RNA	2.438	-5.793 to 10.67	No	ns	0.7333
BNT162b2	No peptide vs. S peptide	-1177	-1314 to -1041	Yes	****	<0.0001
	Control RNA vs. S RNA	-1311	-1448 to -1175	Yes	****	<0.0001
BNT162c2	No peptide vs. S peptide	-1174	-1293 to -1056	Yes	****	<0.0001
	Control RNA vs. S RNA	-1171	-1290 to -1053	Yes	****	<0.0001

Flow cytometry – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 7

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD8^+$ T cells	Control vs. BNT162a1	-1.250	-3.386 to 0.8864	No	ns	0.3001
	Control vs. BNT162b1	-1.225	-3.361 to 0.9114	No	ns	0.3130
	Control vs. BNT162b2	-10.63	-13.87 to -7.377	Yes	****	<0.0001
	Control vs. BNT162c2	-0.4321	-3.794 to 2.930	No	ns	0.9348
$CD4^+$ T cells	Control vs. BNT162a1	1.188	-0.9863 to 3.361	No	ns	0.3445
	Control vs. BNT162b1	1.425	-0.7488 to 3.599	No	ns	0.2302
	Control vs. BNT162b2	11.06	7.473 to 14.65	Yes	****	<0.0001
	Control vs. BNT162c2	-0.7571	-4.473 to 2.959	No	ns	0.8463

T_{FH} cells	Control vs. BNT162a1	-0.04500	-0.1701 to 0.08005	No	ns	0.6086
	Control vs. BNT162b1	-0.3726	-0.4977 to -0.2476	Yes	****	<0.0001
	Control vs. BNT162b2	-0.3828	-0.7397 to -0.02579	Yes	*	0.0350
	Control vs. BNT162c2	-1.190	-1.560 to -0.8208	Yes	****	<0.0001
B cells	Control vs. BNT162a1	7.813	5.540 to 10.08	Yes	****	<0.0001
	Control vs. BNT162b1	7.900	5.628 to 10.17	Yes	****	<0.0001
	Control vs. BNT162b2	14.64	9.503 to 19.77	Yes	****	<0.0001
	Control vs. BNT162c2	9.921	4.607 to 15.24	Yes	***	0.0005

Related to Figure 8

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD44^+CD38^+PD1^+$ $CD8^+$ T cells	Control vs. BNT162a1	-0.2575	-1.951 to 1.436	No	ns	0.9102
	Control vs. BNT162b1	-6.015	-7.708 to -4.322	Yes	****	<0.0001
	Control vs. BNT162b2	-28.05	-33.72 to -22.37	Yes	****	<0.0001
	Control vs. BNT162c2	-1.344	-7.222 to 4.533	No	ns	0.8115
$ICOS^+$ $CD8^+$ T cells	Control vs. BNT162a1	-0.8663	-3.073 to 1.341	No	ns	0.5578
	Control vs. BNT162b1	-8.401	-10.61 to -6.194	Yes	****	<0.0001
	Control vs. BNT162b2	-40.48	-47.07 to -33.89	Yes	****	<0.0001
	Control vs. BNT162c2	-4.713	-11.54 to 2.109	No	ns	0.1998
$ICOS^+$ $CD4^+$ T cells	Control vs. BNT162a1	-0.5650	-1.427 to 0.2973	No	ns	0.2304
	Control vs. BNT162b1	-2.551	-3.414 to -1.689	Yes	****	<0.0001
	Control vs. BNT162b2	-2.981	-5.174 to -0.7890	Yes	**	0.0078
	Control vs. BNT162c2	1.218	-1.051 to 3.488	No	ns	0.3555
$ICOS^+$ Tfh cells	Control vs. BNT162a1	-10.11	-18.24 to -1.987	Yes	*	0.0143
	Control vs. BNT162b1	-26.43	-34.55 to -18.30	Yes	****	<0.0001
	Control vs. BNT162b2	-12.49	-21.24 to -3.733	Yes	**	0.0054
	Control vs. BNT162c2	-19.20	-27.95 to -10.45	Yes	****	<0.0001

Related to Figure 9

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
$CD8^+$ T cells	Control vs. BNT162a1	-337552	-833529 to 158426	No	ns	0.2084
	Control vs. BNT162b1	-420848	-916825 to 75130	No	ns	0.1019
	Control vs. BNT162b2	-683950	-1112484 to -255415	Yes	**	0.0021
	Control vs. BNT162c2	-75551	-504086 to 352984	No	ns	0.8818
$CD4^+$ T cells	Control vs. BNT162a1	-749301	-1913693 to 415091	No	ns	0.2411
	Control vs. BNT162b1	-1246977	-2411369 to -82585	Yes	*	0.0352
	Control vs. BNT162b2	-1850559	-2886016 to -815102	Yes	***	0.0007
	Control vs. BNT162c2	-216563	-1252020 to 818895	No	ns	0.8389
T_{FH} cells	Control vs. BNT162a1	-2366	-7903 to 3171	No	ns	0.5051
	Control vs. BNT162b1	-14242	-19780 to -8705	Yes	****	<0.0001
	Control vs. BNT162b2	-46173	-60706 to -31640	Yes	****	<0.0001
	Control vs. BNT162c2	-4251	-18783 to 10282	No	ns	0.7150
T_H1 cells	Control vs. BNT162a1	-7820	-18193 to 2552	No	ns	0.1541
	Control vs. BNT162b1	-13043	-23416 to -2671	Yes	*	0.0134
	Control vs. BNT162b2	-2268	-4564 to 28.31	No	ns	0.0531
	Control vs. BNT162c2	297.1	-1999 to 2593	No	ns	0.9339

Related to Figure 10

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
B cells	Control vs. BNT162a1	-415609	-1302980 to 471763	No	ns	0.4459
	Control vs. BNT162b1	-1266693	-2154065 to -379321	Yes	**	0.0053
	Control vs. BNT162b2	-761299	-1089182 to -433417	Yes	****	<0.0001
	Control vs. BNT162c2	26360	-301523 to 354242	No	ns	0.9738
Plasma cells	Control vs. BNT162a1	-244.3	-18256 to 17768	No	ns	0.9992
	Control vs. BNT162b1	-20130	-38142 to -2117	Yes	*	0.0278

	Control vs. BNT162b2	-4115	-9265 to 1034	No	ns	0.1206
	Control vs. BNT162c2	-79.67	-5383 to 5223	No	ns	0.9989
Class switched B cells	Control vs. BNT162a1	-69656	-150840 to 11528	No	ns	0.0977
	Control vs. BNT162b1	-280116	-361300 to -198931	Yes	****	<0.0001
	Control vs. BNT162b2	-509776	-672575 to -346977	Yes	****	<0.0001
	Control vs. BNT162c2	-18446	-186106 to 149214	No	ns	0.9444
Germinal center B cells	Control vs. BNT162a1	-66066	-140887 to 8755	No	ns	0.0871
	Control vs. BNT162b1	-267264	-342085 to -192443	Yes	****	<0.0001
	Control vs. BNT162b2	-509776	-673052 to -346500	Yes	****	<0.0001
	Control vs. BNT162c2	-18261	-186412 to 149889	No	ns	0.9457
IgG1$^+$ Germinal center B cells	Control vs. BNT162a1	-17771	-40867 to 5325	No	ns	0.1444
	Control vs. BNT162b1	-67436	-90532 to -44340	Yes	****	<0.0001
	Control vs. BNT162b2	-152191	-198806 to -105575	Yes	****	<0.0001
	Control vs. BNT162c2	2178	-45830 to 50185	No	ns	0.9902
IgG2a$^+$ Germinal center B cells	Control vs. BNT162a1	-52.50	-205.8 to 100.8	No	ns	0.6362
	Control vs. BNT162b1	-285.0	-438.3 to -131.7	Yes	***	0.0005
	Control vs. BNT162b2	-459.6	-678.2 to -240.9	Yes	***	0.0003
	Control vs. BNT162c2	-31.33	-256.5 to 193.9	No	ns	0.9132

Related to Figure 11

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
T_{FH} cells	Control vs. BNT162a1	-943.6	-8833 to 6946	No	ns	0.9431
	Control vs. BNT162b1	-9561	-17451 to -1671	Yes	*	0.0170
	Control vs. BNT162b2	-47817	-74515 to -21120	Yes	***	0.0007
	Control vs. BNT162c2	35.88	-26661 to 26733	No	ns	>0.9999
Germinal center B cells	Control vs. BNT162a1	14390	-129166 to 157946	No	ns	0.9596
	Control vs. BNT162b1	-382477	-526032 to -238921	Yes	****	<0.0001
	Control vs. BNT162b2	-1601371	-2093312 to -1109430	Yes	****	<0.0001

	Control vs. BNT162c2	-74428	-566369 to 417514	No	ns	0.9111
Class switched B cells	Control vs. BNT162a1	-228955	-468753 to 10843	No	ns	0.0622
	Control vs. BNT162b1	-476506	-716304 to -236708	Yes	***	0.0002
	Control vs. BNT162b2	-1445088	-1887202 to -1002973	Yes	****	<0.0001
	Control vs. BNT162c2	-60963	-503077 to 381151	No	ns	0.9254

Related to Figure 12

Group	Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IFNγ$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-1560	-18564 to 15444	No	ns	0.9660
	Control vs. BNT162b1	-77611	-94615 to -60607	Yes	****	<0.0001
	Control vs. BNT162b2	-140659	-195692 to -85625	Yes	****	<0.0001
	Control vs. BNT162c2	-79418	-134451 to -24384	Yes	**	0.0049
IL-2$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-382.5	-2638 to 1873	No	ns	0.8899
	Control vs. BNT162b1	-9429	-11684 to -7174	Yes	****	<0.0001
	Control vs. BNT162b2	-5903	-10507 to -1298	Yes	*	0.0117
	Control vs. BNT162c2	-406.1	-5010 to 4198	No	ns	0.9685
TNF$^+$ CD8$^+$ T cells	Control vs. BNT162a1	-591.5	-2201 to 1018	No	ns	0.5965
	Control vs. BNT162b1	-4292	-5901 to -2683	Yes	****	<0.0001
	Control vs. BNT162b2	-1403	-2898 to 91.18	No	ns	0.0670
	Control vs. BNT162c2	351.1	-1143 to 1846	No	ns	0.8023
IFNγ$^+$ CD4$^+$ T cells	Control vs. BNT162a1	19.00	-1350 to 1388	No	ns	0.9992
	Control vs. BNT162b1	-3941	-5310 to -2572	Yes	****	<0.0001
	Control vs. BNT162b2	995.9	-2046 to 4038	No	ns	0.6600
	Control vs. BNT162c2	1282	-1760 to 4324	No	ns	0.5140
IL-2$^+$ CD4$^+$ T cells	Control vs. BNT162a1	-1472	-2850 to -92.56	Yes	*	0.0359
	Control vs. BNT162b1	-2276	-3655 to -897.3	Yes	**	0.0016
	Control vs. BNT162b2	-3755	-8093 to 583.6	No	ns	0.0944
	Control vs. BNT162c2	-774.8	-5113 to 3564	No	ns	0.8790

IL-4$^+$ CD4$^+$ T cells	Control vs. BNT162a1	-377.1	-1961 to 1207	No	ns	0.7977
	Control vs. BNT162b1	-520.3	-2104 to 1064	No	ns	0.6583
	Control vs. BNT162b2	-654.0	-1345 to 36.86	No	ns	0.0647
	Control vs. BNT162c2	-71.88	-762.7 to 619.0	No	ns	0.9566
IFNγ$^+$ T_{FH} cells	Control vs. BNT162a1	70.25	-110.7 to 251.2	No	ns	0.5643
	Control vs. BNT162b1	-134.9	-315.8 to 46.04	No	ns	0.1597
	Control vs. BNT162b2	-147.3	-267.6 to -26.93	Yes	*	0.0160
	Control vs. BNT162c2	-19.63	-139.9 to 100.7	No	ns	0.8976

Multiplex protein quantification – details on statistical analysis performed with GraphPad Prism 8.

Related to Figure 13

Group	Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IFNγ	Control (mCorVac#15)	-0,9662	-969,8 to 967,8	No	ns	>0,9999
	BNT162a1	-202,2	-1171 to 766,6	No	ns	0,9330
	BNT162b1	-2025	-2994 to -1056	Yes	****	<0,0001
	Control	-3,160	-20,63 to 14,31	No	ns	0,9548
	BNT162b2	-4780	-4798 to -4763	Yes	****	<0,0001
	BNT162c2	-4794	-4811 to -4776	Yes	****	<0,0001
IL-2	Control	1,260	-12,20 to 14,72	No	ns	0,9932
	BNT162a1	-27,52	-40,98 to -14,06	Yes	****	<0,0001
	BNT162b1	-27,35	-40,81 to -13,90	Yes	****	<0,0001
	Control	14,00	-15,79 to 43,79	No	ns	0,5549
	BNT162b2	-81,77	-111,6 to -51,98	Yes	****	<0,0001
	BNT162c2	-63,07	-92,86 to -33,28	Yes	****	<0,0001
IL-4	Control	0,000	-9,014 to 9,014	No	ns	>0,9999
	BNT162a1	-3,488	-12,50 to 5,526	No	ns	0,6953
	BNT162b1	-16,09	-25,11 to -7,079	Yes	***	0,0004
	Control	-4,335	-26,27 to 17,60	No	ns	0,9423

	BNT162b2	-101,9	-123,9 to -79,98	Yes	****	<0,0001
	BNT162c2	-29,45	-51,39 to -7,513	Yes	**	0,0067
IL-5	n/a	n/a	n/a	n/a	n/a	n/a
	n/a	n/a	n/a	n/a	n/a	n/a
	n/a	n/a	n/a	n/a	n/a	n/a
	Control	3,553e-015	-7,207 to 7,207	No	ns	>0,9999
	BNT162b2	-24,75	-31,95 to -17,54	Yes	****	<0,0001
	BNT162c2	-6,075	-13,28 to 1,132	No	ns	0,1160
IL-18	Control	-0,4713	-355,8 to 354,9	No	ns	>0,9999
	BNT162a1	-667,2	-1023 to -311,9	Yes	***	0,0002
	BNT162b1	-1914	-2270 to -1559	Yes	****	<0,0001
	Control	-54,48	-627,7 to 518,7	No	ns	0,9929
	BNT162b2	-6801	-7374 to -6228	Yes	****	<0,0001
	BNT162c2	-7150	-7723 to -6576	Yes	****	<0,0001
GM-CSF	Control	0,000	-5,859 to 5,859	No	ns	>0,9999
	BNT162a1	-3,166	-9,025 to 2,693	No	ns	0,4398
	BNT162b1	-21,55	-27,41 to -15,69	Yes	****	<0,0001
	Control	-2,842e-014	-20,39 to 20,39	No	ns	>0,9999
	BNT162b2	-146,4	-166,8 to -126,0	Yes	****	<0,0001
	BNT162c2	-78,13	-98,53 to -57,74	Yes	****	<0,0001
n/a measured values < lower limit of quantification						

9.11 List of attachments

Attachment I includes the following raw data sets:

- Attachment_001_CorVac#15_phenotypic_analysis_blood_Freq_Report
- Attachment_002_CorVac#16_phenotypic_analysis_blood_Freq_Report
- Attachment_003_CorVav#15_phenotypic_analysis_Spleen_Freq_Report
- Attachment_004_CorVac#16_phenotypic_analysis_Spleen_Freq_Report
- Attachment_005_CorVav#15_phenotypic_analysis_LN_Freq_Report
- Attachment_006_CorVav#16_phenotypic_analysis_LN_Freq_Report
- Attachment_007_CorVac#15_phenotypic_analysis_Spleen_Counts_Report
- Attachment_008_CorVac#16_phenotypic_analysis_Spleen_Counts_Report
- Attachment_009_CorVav#15_phenotypic_analysis_LN_Counts_Report

- Attachment_010_CorVav#16_phenotypic_analysis_LN_Counts_Report
- Attachment_011_CorVac#15_phenotypic_analysis_Spleen_gMFI_Report
- Attachment_012_CorVac#16_phenotypic_analysis_Spleen_gMFI_Report
- Attachment_013_CorVac#15_phenotypic_analysis_LN_gMFI_Report
- Attachment_014_CorVav#16_phenotypic_analysis_LN_gMFI_Report
- Attachment_015_CorVac#15_myeloid_Spleen_Freq_Report
- Attachment_016_CorVac#16_myeloid_Spleen_Freq_Report
- Attachment_017_CorVac#15_myeloid_Spleen_Counts_Report
- Attachment_018_CorVac#16_myeloid_Spleen_Counts_Report
- Attachment_019_CorVac#15_functional_analysis_Spleen_Freq_Report
- Attachment_020_CorVac#16_functional_analysis_Spleen_Freq_Report
- Attachment_021_CorVac#15_functional_analysis_LN_Freq_Report
- Attachment_022_CorVac#16_functional_analysis_LN_Freq_Report
- Attachment_023_CorVac#15_functional_analysis_Spleen_Counts_Report
- Attachment_024_CorVac#16_functional_analysis_Spleen_Counts_Report
- Attachment_025_CorVac#15_functional_analysis_LN_Counts_Report
- Attachment_026_CorVac#16_functional_analysis_LN_Counts_Report
- Attachment_027_CorVav#15_B-cell_Spleen_Freq_Report
- Attachment_028_CorVav#16_B-cell_Spleen_Freq_Report
- Attachment_029_CorVav#15_B-cell_LN_Freq_Report
- Attachment_030_CorVav#16_B-cell_LN_Freq_Report
- Attachment_031_CorVac#15_B-cell_Spleen_Counts_Report
- Attachment_032_CorVac#16_B-cell_Spleen_Counts_Report
- Attachment_033_CorVac#15_B-cell_LN_Counts_Report
- Attachment_034_CorVac#16_B-cell_LN_Counts_Report
- Attachment_035_CorVac#15_memB-cell_Spleen_Counts_Report
- Attachment_036_CorVac#16_memB-cell_Spleen_Counts_Report
- Attachment_037_CorVac#15_memB-cell_LN_Counts_Report
- Attachment_038_CorVac#16_memB-cell_LN_Counts_Report

Attachment II includes all gating strategies used for the analysis of flow cytometry data

Attachment III includes the following raw data sets:

- Attachment_039_CorVac#15_xCelligence_Report
- Attachment_040_CorVac#16_xCelligence_Report

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R&D DATA REPORT No. R-20-0211

In Vitro Expression of BNT162b2 Drug Substance and Drug Product

Version 02
Date: 17 SEP 2020

Reported by (b) (6)

Test item: BNT162b2
Key words: COVID19, modRNA, ATM material, Western blot, immunofluorescence, FACS

This R&D report consists of 22 pages.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ATM	Animal trial material
BNT162b2	Investigational vaccine in this study (DP)
BNT162b2-RNA	Investigational vaccine in this study (DS)
COVID-19	Coronavirus disease emerged in 2019
DNA	Deoxyribonucleic acid
DP	Drug product
DS	Drug substance
ER	Endoplasmic reticulum
FACS	Fluorescence-activated cell sorting
GFP	Green fluorescent protein
HEK	Human embryonic kidney
modRNA	modified RNA
PAGE	Polyacrylamide gel electrophoresis
RBD	Receptor binding domain
RNA	Ribonucleic acid
S protein	Spike protein
S1	Subdomain 1 of the S protein
SARS-CoV-2	Severe acute respiratory syndrome-Coronavirus-2
saRNA	self-amplifying RNA
SDS	Sodium dodecyl sulfate
SOP	Standard operating procedure
uRNA	unmodified RNA
V9	Antigen variant of the generated variants of the S protein

RESPONSIBILITIES

Person responsible for the study:	(b) (6) (b) (6) BioNTech RNA Pharmaceuticals GmbH	18 Sep 2020 Date
Author:	(b) (6) (b) (6) BioNTech RNA Pharmaceuticals GmbH	18 Sep 2020 Date
Reviewer:	(b) (6) (b) (6) BioNTech RNA Pharmaceuticals GmbH	18 Sep 2020 Date
QA representative:	(b) (6) BioNTech SE	22 Sep 2020 Date

Meaning of the signatures:

Person responsible for the study: I am responsible for the content of the R&D report and confirm that it represents an accurate record of the results. This study was performed according to the SOPs and methods as well as the rules and regulations described in the report.

Author: I am the author of this document.

Reviewer: I reviewed the R&D report and confirm that this document complies with the scientific and technical standards and requirements.

QA representative: I confirm that this document complies with the relevant quality assurance requirements.

1 SUMMARY

BioNTech is developing RNA-based vaccines designed to protect against the novel coronavirus disease that emerged in 2019 (COVID-19). The project involves testing of three RNA platforms, namely non-modified uridine containing mRNA (uRNA), nucleoside modified mRNA (modRNA) and self-amplifying RNA (saRNA), which are under development at BioNTech, with the spike protein (S protein) of the novel Coronavirus (SARS-CoV-2) as the viral antigen.

In the present study, antigen expression was evaluated of the current clinical lead candidate, BNT162b2. BNT162b2 is a nucleoside-modified mRNA (modRNA) encoding the antigen variant 9 (V9) of the S protein. BNT162b2 was analyzed both as drug substance (DS; BNT162b2-RNA), and as LNP-formulated drug product (DP; BNT162b2) in HEK293T cells. DS was transfected into cells using a commercial transfection reagent.

Antigen expression was investigated and confirmed by Western blot analysis of HEK293T cell lysates of cells that had been transfected with DS BNT162b2-RNA.

Transfection frequencies and expression of the antigen in both DS and DP, BNT162b2-RNA and BNT162b2, were evaluated by FACS analysis of transfected HEK293T cells. Both, BNT162b2-RNA and BNT162b2 revealed high frequencies of transfected cells, with BNT162b2 cells showing slightly higher transfection frequencies compared to BNT162b2-RNA transfection. There were no differences in cell viability after transfection with BNT162b2-RNA or BNT162b2 when comparing to non-transfected cells.

In addition, fluorescence co-staining was performed with an endoplasmic reticulum (ER) marker and an antibody recognizing the S1 protein subunit to evaluate the correct antigen localization using DS BNT162b2-RNA-transfected HEK293T cells. Co-localization of the antigen expressed by DS encoding the full length S protein with an ER marker was confirmed.

(b) (6)		18Sep2020
GmbH	BioNTech RNA Pharmaceuticals	Date

2 GENERAL INFORMATION

2.1 Sponsor and Test Facilities

Sponsor

BioNTech RNA Pharmaceuticals GmbH
An der Goldgrube 12
55131 Mainz
Germany

Test Facilities

BioNTech RNA Pharmaceuticals GmbH
An der Goldgrube 12
55131 Mainz
Germany

(b) (4)

2.2 Participating Personnel

Responsible person: (as defined in SOP-100-024)	(b) (6) BioNTech RNA Pharmaceuticals GmbH An der Goldgrube 12 55131 Mainz
Author:	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter: Western blot, FACS	(b) (6) BioNTech RNA Pharmaceuticals GmbH
Experimenter: Immunofluorescence	(b) (6), (b) (4)

2.3 Study Dates

Start of experiments: 22 JUN 2020

Completion of experiments: 14 AUG 2020

2.4 Guidelines and Regulations

All experiments are executed in accordance with the existing standard operating procedures and described processes from BioNTech SE. Applicable documents are listed below.

- SOP-020-009 Ansetzen von Medien und Zusätzen für die Zellkultur
- SOP-030-038 Standardisierte Kultivierung von Zellen
- SOP-030-039 Zellzahlbestimmung mittels Neubauer Zählkammer
- SOP-030-117 Durchführung einer SDS Polyacrylamid Gelelektrophorese (SDS-PAGE)
- LA-50-255-000 Direkte / Indirekte Immunfluoreszenzfärbung

2.5 Changes and Deviations

Not applicable. There is no formal R&D plan available.

2.6 Documentation and Archive

Study plans and reports are stored and archived according to SOP-100-003 Archiving of Paper-Based Documents.

Raw data and evaluated data are saved at

- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\04_in vitro\CorVac_IDV.invitro#050_b1_b2_b3c_DS_and_DP_IVE
- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\04_in vitro\CorVac_IDV.invitro#059_HEK_IF_microscopy_at(b) (4)
- P:\BioNTechRNA\RN9391R00_CoV-VAC\04_Preclinic\04_in vitro\CorVac_IDV.invitro#064_mod9-WB_IVE
- Lab book MeGl, No. 2034
- Lab book MeGl, No. 2035

3 MATERIALS AND METHODS

3.1 Test Item

BNT162b2-RNA (ATM RNA): For CoA see Appendix 1: Certificates of Analysis.

BNT162b2 (ATM LNP): For CoA see Appendix 1: Certificates of Analysis.

- RNA batch: RNA-RF200321-06, 97.5% integrity
- Polymun batch RBP020.2 LNP with the lot: CoVVAC/270320

3.2 Control Item

- Modified RNA GFP, p4.AGA_eGFP, RNA_RF200309_01c, 96% integrity

3.3 Test System

- *In vitro* test system, cell culture, HEK293T cells

3.4 Materials

Table 1: Equipment

Product name	Provider
Microscope IX53	Olympus Life Science
Microscope SP8	Leica
HeraCell 150i incubator	Thermo Fisher Scientific
Vortexer	Neolab
Biological Safety Cabinet HeraSafe2020	Thermo Fisher Scientific
Trans-Blot Turbo Transfer System	Bio-Rad
Gel Dokumentation System ChemiDoc MP Imaging system, Detektor Supercooled CCD -30 °C, Pixel (Graustufen) 65535	Bio-Rad
Vacuum pump BVC-vacuu-control	Vacuubrand
Pipette Eppendorf Research, 10-100 µL	Eppendorf
Pipette Eppendorf Research, 100-1,000 µL	Eppendorf
Pipette Eppendorf Research, 20-200 µL	Eppendorf
Centrifuge 5810R	Eppendorf
FACSCanto II	BD
Leica Application Suite LAS-X Version 3.1.5.16308	Leica
FlowJo software version 10.6.2	FlowJo LLC, BD Biosciences
Image Lab software version 5.0.	Bio-Rad

Table 2: Consumables

Product name	Application/specification	Article no.	Provider
12-well plates	Tissue culture	665180	Greiner-bio-one
96-well plates	Tissue culture	650101	Greiner-bio-one
Safe-lock tubes	1.5 mL	0030120.086	Eppendorf
Tubes	15 mL	188271	Greiner-bio-one
Filter tips	20-200 µL	30077555	Eppendorf
Filter tips	100-1,000 µL	10212393	Eppendorf
Filter tips	0.1-10 µL	30077512	Eppendorf
Aspirating pipets	2 mL w/o plug	710183	Greiner-bio-one
Serological pipets	10 mL	607160	Greiner-bio-one

Table 3: Reagents

Product name	Application/specification	Article no.	Provider
RiboJuice	Transfection reagents	TR-1013	Merck Millipore
FBS superior	Fetal bovine serum	81D2925	Biochrom GmbH
DPBS	No calcium, no magnesium	14190-094	Thermo Fisher Scientific
StemPro™ Accutase™	Cell Dissociation Reagent	A11105-01	Thermo Fisher Scientific
Opti-MEM GlutaMAX	Reduced serum medium	51985034	Thermo Fisher Scientific
EDTA	0.5 M	03690-100ML	Sigma-Aldrich
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate	Cell culture	31966047	Thermo Fisher Scientific
BSA	Bovine serum albumin	GAUBSA01-64	Eurobio
Triton X-100	Immunofluorescence	X100-100ML	Sigma Aldrich
Hoechst	1:5,000	H3570	Life Technologies
PFA, 32%	Fixation	15714-5	Electron Microscopy
ImmoMount media	Mounting media	9990402	Life Technologies
4–15% Mini-PROTEAN® TGX™ Precast Protein Gels	Polyacrylamide gel	4561083	Bio-Rad
cOmplete™ ULTRA Tablets, Mini, EASYpack	Protease Inhibitor Cocktail	5892970001	Roche
Transfermembran Amersham™ Protran® NC	Nitrocellulose membrane	4675.1	Carl Roth
Color Prestained Protein Standard, Broad Range (11–245 kDa)	Molecular marker	P7712	New England BioLabs
10x Tris/Glycine/SDS	Running Buffer	1610772	Bio-Rad
4 x Laemmli Sample Buffer	Western blot	1610747	Bio-Rad
DTT	Western blot	A2948,0025	PanReac AppliChem

Product name	Application/specification	Article no.	Provider
Fixable Viability Dye eFluor™ 450	FACS	65-0863-14	eBioscience
Fixation Buffer	FACS	420801	Biolegend
Permeabilization Buffer (10X)	FACS	00-8333-56	eBioscience
Ethanol	Western blot	5054.4	Carl Roth
Nonfat dried milk powder	Western blot	A0830,1000	AppliChem
Tween-20	Western blot	9127.1	Carl Roth
Pierce™ ECL Western Blotting Substrate	Chemiluminescent substrate	32209	Thermo Fisher Scientific
Tris	Western blot	3170.2	Carl Roth
Glycin	Western blot	3790.2	Carl Roth
SDS	Western blot	0183.1	Carl Roth
NaCl	Western blot	9265.2	Carl Roth
Sodium deoxycholate	Western blot	S1827-100G	Sigma Aldrich
EDTA	Western blot	8040.3	Carl Roth

Table 4: Antibodies and recombinant protein controls

Product name	Dilution	Article no.	Provider
SARS-CoV Spike S1 Subunit Protein Antibody, Rabbit PAb	1:100 (IF); 1:1,000 (WB)	40150-RP01	Sino Biological
SARS-CoV-2 (2019-nCoV) Spike Antibody, Rabbit Mab	1:400 (FACS)	40150-R007	Sino Biological
Alexa Fluor™ 647 Antibody Labeling Kit	N/A	A20186	ThermoFisher Scientific
Concanavalin A, Alexa Fluor™ 594 Conjugate	1:100 (IF)	C11253	Invitrogen
Lectin GS-II From Griffonia simplicifolia, Alexa Fluor™ 594 Conjugate	1:100	L21416	Invitrogen
Alexa Fluor® 488 AffiniPure Goat Anti-Rabbit IgG (H+L)	1:400 (IF)	111-545-003	Jackson ImmunoResearch
Anti-Rabbit IgG (whole molecule)–Peroxidase antibody produced in goat	1:2,000 (WB)	A0545	Sigma Aldrich
NCP-CoV(2019-nCoV) Spike Protein (S1 Subunit, His Tag)	N/A	40591-V08H	Sino Biological

3.5 Methods

Western blot and FACS assays were performed to analyze antigen expression; immunofluorescence experiments were performed to assess correct localization in cells.

3.5.1 Study Design

The aim of this study was to analyze the expression of BNT162b2 DS and DP in HEK293T cells as a surrogate for a mammalian cell culture system. HEK293T cells were transfected with DS BNT162b2-RNA using a commercial transfection reagent or with DP BNT162b2 to express the S protein of SARS-CoV-2 as the viral antigen. Transfected HEK293T cells were allowed to express the S protein for 18 h before analysis.

Expression of DS BNT162b2-RNA was analyzed using Western blot to confirm expression.

FACS analysis was performed in triplicates in permeabilized cells to assess transfection frequencies and viability of cells transfected with either the DS BNT162b2-RNA, or with the DP BNT162b2.

Finally, immunofluorescence microscopic analysis was used on cells transfected with the DS BNT162b2-RNA to assess processing of the expressed protein in the endoplasmic reticulum (ER). Since the S protein contains a transmembrane domain it is expressed on the cell surface, and therefore processing in the ER was presumed.

3.5.2 Transfection of RNA Constructs in HEK293T Cells

HEK293T cells were seeded in 12-well plates with a cell number of 2×10^5 per well one day before transfection or with a cell number of 4×10^5 per well 6 h before transfection in DMEM with 10% FBS. For immunofluorescence experiments, HEK293T cells were seeded in 12-well plates with cover slips previously coated in collagen with a cell number of 2×10^5 per well one day before transfection in DMEM with 10% FBS.

Cells were transfected with DS BNT162b2-RNA using Ribojuice according to the manufacturer's protocol. As a transfection control, a modRNA construct encoding GFP was used. Briefly, 1 µg of RNA was diluted in Opti-MEM and mixed with transfection reagents. After an incubation of 4 min, 100 µL of the mixture was applied to the cells, mixed gently, and incubated at 37°C/5% CO_2 for 18 h. For FACS analysis DP BNT162b2 was transfected by diluting 1 µg of DP in 100 µl OptiMEM. The mixture was applied to the cells, mixed gently and centrifuged by 500×g for 5 min at room temperature before incubating at 37°C/5% CO_2 for 18 h.

Before proceeding with subsequent analyses, cells transfected with GFP were examined microscopically for successful transfection. Cells transfected with DS or DP encoding for the antigen were either harvested for Western blot analysis or prepared for subsequent immunofluorescence or FACS analysis

3.5.3 Western Blot Analysis

Western blot analyses were used to evaluate whether the designed constructs were expressed in HEK293T cells.

HEK293T cells were washed with PBS and detached from the well plate. Cells were collected in a 1.5 ml Eppendorf tube, centrifuged for 5 min at 300×g/4°C. Supernatants were discarded and the cell pellet was dissolved in 40 µL RIPA buffer (20 mM Tris, 0 15 M NaCl, 1% Triton X 100, 1% odium deo ycholate, 0 1% SDS, 10 mM EDTA) with protease inhibitors and incubated for 30 min on ice. 13.3 µL 4x Laemmli sample buffer with 10% DTT was added to the Eppendorf tubes and samples were heated for 5 min at 95°C. Recombinant protein controls were treated equally with 4x Laemmli sample buffer/10% DTT diluted in PBS to achieve a 1x dilution. Afterwards, gels were loaded with 25 µL of the samples and 3 µL of a marker. Gel electrophoresis was performed with Tris/glycine/SDS running buffer at 120 V. Proteins were transferred on a nitrocellulose membrane for 30 min at 25 V (max. 1 A) using transfer buffer (43 mM Tris, 35 mM glycine, 10% ethanol). The membranes were subsequently washed with PBS/0.1% Tween-20, blocked for 1 h with blocking buffer (PBS, 0.1% Tween-20, 5% nonfat dried milk powder) and incubated with the primary antibody in blocking buffer overnight at 4°C.

After incubation, membranes were washed with PBS/0.1% Tween-20 before incubation with the secondary antibody for 1 h at room temperature, and washed again before developing with chemiluminescent substrate and subsequent analysis on a BioRad ChemiDoc system.

3.5.4 FACS Analysis

To assess transfection frequencies of DS BNT162b2-RNA transfected cells using a commercial transfection reagent or DP BNT162b2 transfected cells, FACS analysis was performed. Cells were transferred to a 96-well plate format and stained with 50 µl fixable viability dye eFluor™ 450 diluted 1:500 for 15 min at room temperature. To remove residual dye, cells were washed with FACS Buffer (1xDPBS, 1% BSA, 1% 0.5M EDTA), centrifuged at 300xg for 5 min at 4°C and fixed with 100 µl Fixation Buffer (Biolegend) for 12 minutes at room temperature. Cells were washed with 1x Permeabilization Buffer (eBioscience) by centrifuging at 500xg for 5 min at 4°C and stained with 50 µl anti-S1 antibody labelled with AF647 diluted 1:400 in 1x permeabilization buffer for 30 min on ice. Afterwards, cells were washed twice with 1x permeabilization buffer, centrifuging at 500xg for 5 min at 4°C. Cells were resuspended in 100 µl FACS buffer before acquisition with a BD FACSCanto II.

3.5.5 Immunofluorescence

Immunofluorescence staining of transfected cells was used to test whether the construct was processed within the endoplasmic reticulum (ER) towards the cell membrane leading to secretion or surface expression.

HEK293T cells were washed twice in PBS and fixed in 4% PFA for 10 min. Afterwards, cells were washed three times for 5 min in PBS, permeabilized in PBS/0.2% Triton X-100 for 5 min and blocked in PBS with 2% BSA and 5% goat sera for 30 min. Cells were then incubated for 1.5 h with the primary antibody in PBS/2% BSA (anti-S1 antibody), washed three times in PBS for 5 min, and incubated with secondary antibodies and conjugated Concanavalin A and Lectin GS-II in PBS/2%BSA for 2 h. Afterwards, cells were stained with Hoechst for 3 min, washed three times in PBS for 5 min and were then mounted on slides and stored at 4°C until analysis. Cells were analyzed with a Leica SP8 confocal microscope.

4 TABLES AND FIGURES

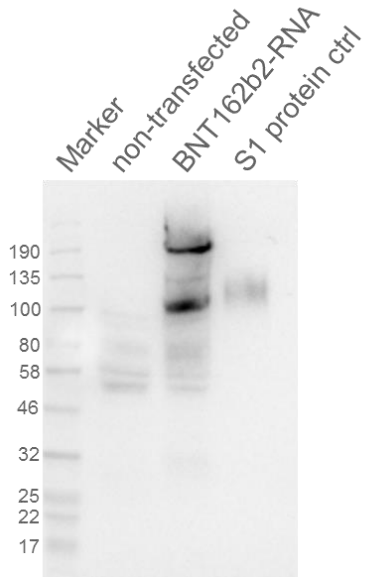

Figure 1: Western blot analysis for detection of BNT162b2 antigen expression

Cells were transfected with DS BNT162b2-RNA, and harvested after 18 h to allow antigen expression. Cells were lysed and lysates were subjected to a sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) system using a 4–15% gradient polyacrylamide gel followed by Western blot analysis. BNT162b2 has a predicted size of 141.14 kDa. A recombinant SARS-CoV-2 S1 Subunit protein (76.5 kDa) was used as a positive control.

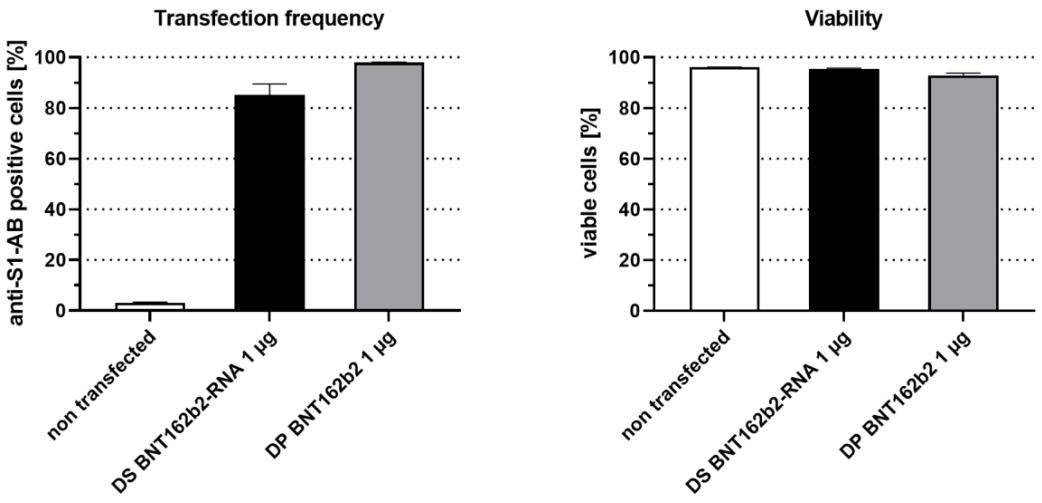

Figure 2: FACS analysis of transfection frequency and cell viability

Cells were transfected with BNT162b2-RNA or BNT162b2. After 18 h in culture, cells were stained with a viability dye, fixed, permeabilized and stained with a monoclonal rabbit antibody recognizing the S1 protein subdomain labelled with AF647. Non-transfected cells were used as a control.

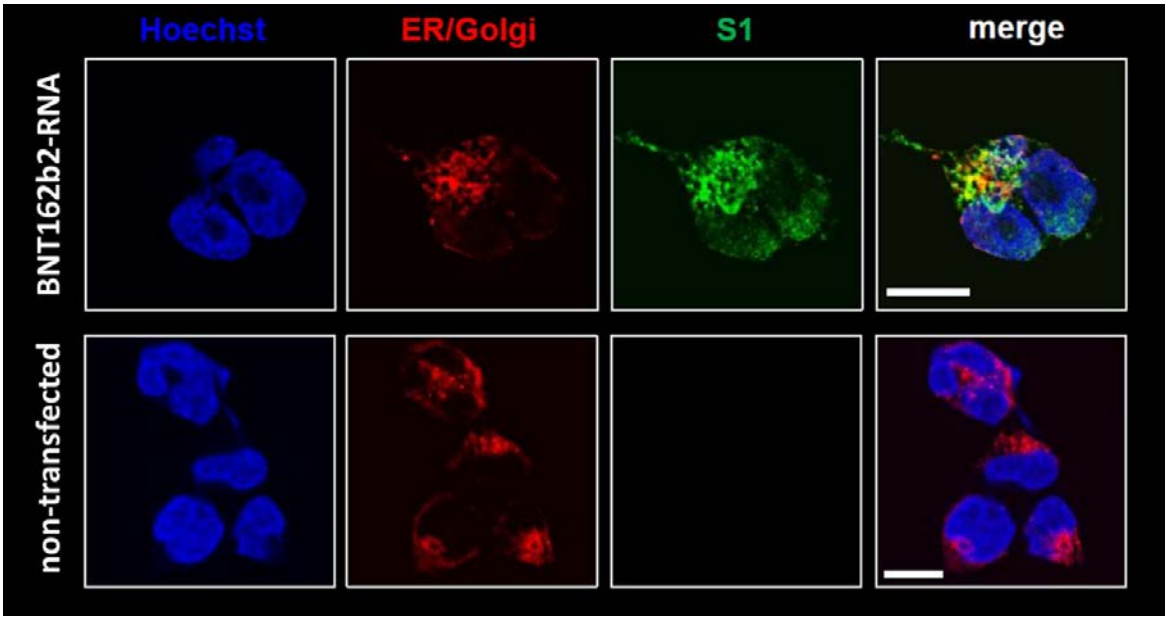

Figure 3: Immunofluorescence staining of transfected cells

Cells were transfected with BNT162b2. After 18 h in culture, cells were fixed and stained for: the ER (Concanavalin A, Alexa Fluor™ 594 conjugate and Lectin GS-II from Griffonia simplicifolia, Alexa Fluor™ 594 conjugate, red), the S1 protein subdomain using a polyclonal antibody (anti-S1 antibody and Alexa Fluor® 488, green) and deoxyr bonucleic acid (DNA) to define the nucleus (Hoechst, blue). The merged colored picture shows the co-localization of the two candidates within the ER (scale: 10 µm). A control, using non-transfected cells, is shown in the bottom row.

5 CONCLUSION

Western blot analysis confirmed the expression and size of the BNT162b2 antigen in cell lysates of HEK293T cells as a surrogate for correct expression in a eukaryotic system.

FACS analysis was performed to assess transfection frequencies of HEK293T cells transfected with either BNT162b2 drug substance (BNT162b2-RNA) or drug product (BNT162b2). Both, BNT162b2-RNA and BNT162b2 led to high frequencies of cells being transfected, with BNT162b2-transfected cells showing slightly higher transfection frequencies compared to BNT162b2-RNA transfected cells using a commercial transfection reagent. There were no differences in cell viability after transfection with BNT162b2-RNA or BNT162b2 when comparing to non-transfected cells.

Furthermore, co-localization of the S protein antigen with an ER marker was detected by immunofluorescence experiments in HEK293T cells expressing BNT162b2-RNA. These results show that the S protein is processed within the ER for surface expression.

6 DOCUMENT HISTORY

Reasons for changes compared to previous version:

Section	Version	Version	Reason for change
2.6	01	02	Specification of lab book numbers
3.4	01	02	Equipment was added
3.4	01	02	Correction of IF staining
3.5.5	01	02	Correction of IF staining
4	01	02	Correction of IF staining

7 REFERENCES

Not applicable.

8 APPENDIX

Appendix 1: Certificates of Analysis

BNT162b2-RNA

BioNTech RNA Pharmaceuticals GmbH

An der Goldgrube 12, 55131 Mainz, Germany
Tel.: +49 (0) 6131-90 84-0, Fax: +49 (0) 6131-90 84-390,
info@biontech.de

BIONTECH
RNA Pharmaceuticals

Report of Results
In vitro transcribed mRNA

Product:	***In vitro* transcribed mRNA RBP020.2 (ATM batch modRNAv09)**
Lot/Batch No.:	RNA-RF200321-06
RNA length:	4283 nt
Media and additives:	10 mM HEPES/0.10 mM EDTA (pH 7.0)
Production date:	19 Mar 2020 (produced by BioNTech RNA Pharmaceuticals GmbH)
Storage:	-30 °C to -15 °C

Test	Result
Content (RNA concentration) Ultraviolet Absorption Spectrophotometry; A_{260}	(b) (4)
Identity (RNA length) Denaturing Agarose Gel Electrophoresis	
RNA integrity Capillary Electrophoresis (Fragment Analyzer, Advanced Analytical)	
Potency *In vitro* translation followed by gel electrophoresis	
pH Potentiometric Determination of pH	
Bacterial Endotoxins LAL-test (Ph. Eur. 2.6.14)	
Residual DNA template Quantitative PCR	
Residual dsRNA Antibody-based limit test	
Osmolality Measurement of depression of freezing point	
Bioburden Microbial examination of non sterile products (Ph. Eur. 2.6.12)	

Remarks:

None.

BNT162b2

POLYMUN SCIENTIFIC
Immunologische Forschung GmbH

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A-3400 Klosterneuburg, Austria
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Fax: +43-2243-25060-399
E-Mail: office@polymun.com
http://www.polymun.com

Non-GMP CoA
Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.2LNP
Lot: CoVVAC/270320

Test	Method
Appearance	Visual Inspection (224/SOP/011)
RNA identity	CE (223/SOP/016)
RNA integrity	CE (223/SOP/016)
RNA content	Ribogreen Assay (221/SOP/018)
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)
ALC-0315 content	HPLC-CAD (222/SOP/044)
ALC-0159 content	HPLC-CAD (222/SOP/044)
DSPC content	HPLC-CAD (222/SOP/044)
Cholesterol content	HPLC-CAD (222/SOP/044)
Particle size (Z_{ave})	Dynamic light scattering (224/SOP/002)
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)
pH	pH (224/SOP/016)
Osmolality	Freezing point depression (224/SOP/009)
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)
Bioburden	Membrane filtration method 225/SOP/001

(b) (4)

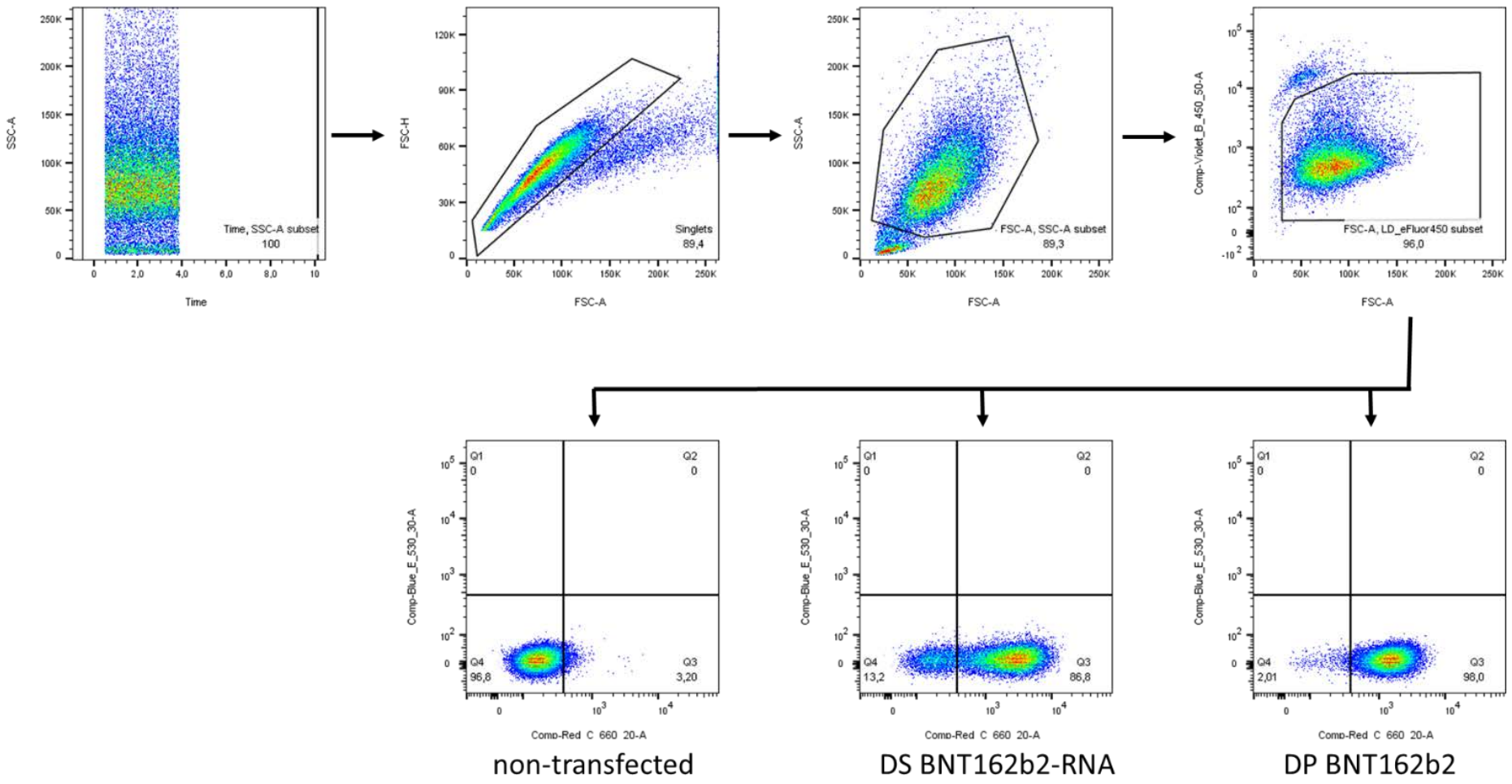
Store at: -70°C

Date: 09.04.20

Date: 09.04.20

(b) (6)

Appendix 2: FACS gating strategy



Title: Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen

Study Number: N/A

Parent Compound Number(s): PF-07302048

Alternative Compound Identifiers: N/A

Pfizer Discovery Sciences
Eastern Point Road
Groton, CT

Title: Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen

PRINCIPAL INVESTIGATOR: (b) (6)

CONTRIBUTING SCIENTIST(S):

(b) (6)

PREPARED BY:

(b) (6)

APPROVED BY:

(b) (6)

Title: Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen

SYNOPSIS

The binding and structural analysis of SARS-CoV-2 P2 S expressed from DNA that encodes the same amino acid sequence as BNT162b2 RNA indicate that the encoded P2 S antigen authentically presents the ACE2 binding site and other epitopes targeted by SARS-CoV-2 neutralizing antibodies.

TABLE OF CONTENTS

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LIST OF FIGURES

Title: Structural and Biophysical Characterization of SARS-CoV-2 Spike Glycoprotein (P2 S) as a Vaccine Antigen

Study Number: N/A

Functional Area: Medicinal Sciences

Test Facility: Pfizer Discovery Sciences, Eastern Point Road, Groton, CT

Study/Testing Initiation Date: 07April2020

Study/Testing Completion Date: 19Aug2020

1. OBJECTIVES

The purpose of this study was to express and characterize the vaccine antigen encoded by BNT162b2.

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). Coronavirus S is a major target of virus neutralizing antibodies and is a key antigen for vaccine development. S is a transmembrane glycoprotein responsible for receptor recognition, attachment to the cell, and viral envelope fusion with a host cell membrane resulting in genome release. While the membrane-proximal S2 is responsible for membrane fusion, the membrane-distal S1, with its receptor-binding domain (RBD), recognizes the host receptor, angiotensin converting enzyme 2 (ACE2) (Zhou et al, 2020). The RBD forms membrane distal “heads” on the S trimer that are connected to the body by a hinge. In the native S, the RBD alternates between an open (up) and closed (down) position. Although potent neutralizing epitopes have been described when the RBD is in the “heads down” closed conformation, the “heads up” receptor accessible conformation exposes a potentially greater breadth of neutralizing antibody targets (Brouwer et al, 2020; Liu et al, 2020; Robbiani et al, 2020).

The glycoprotein encoded by the vaccine candidate BNT162b2 includes two amino acid substitutions to proline (P2 S) locking the transmembrane protein in an antigenically optimal prefusion conformation (Pallesen et al, 2017; Wrapp et al, 2020). The P2 S antigen was expressed from DNA and characterized for structure and binding to human ACE2 and SARS-CoV-2 neutralizing antibodies.

3. MATERIALS AND METHODS

3.1. Flow Cytometry Analysis of Binding to Cell Surface-Expressed P2 S

A modified pcDNA3.1 Zeo (+) construct encoding the P2 S antigen under control of a CAG promoter was expressed in Expi293F cells as per the supplied Expifectamine 293 Transfection Kit protocol.

Cells were collected 48 hr post transfection, washed with TBS buffer, and used at 4-5 x 10^4 for each condition. Cells were incubated for 1 hr at room temperature (RT) in TBS + 4% BSA + 0.01 mg/mL 7-AAD to detect non-viable cells and with either (i) 1:100 FITC-labeled anti-6xHis plus 10 nM His-tagged human ACE2 peptidase domain (ACE2-PD); (ii) 100 nM Alexa-488 labeled anti-Rabbit IgG Fab plus either 33 nM anti-SARS-CoV-2 Spike RBD (αRBD, Sino Biological 40592-T62), anti-SARS-Cov-2 Spike S1 (αS1, Sino Biological 40150-R007), or anti-SARS Spike S2 (αS2, Novus NB100-56578); or (iii) 100 nM Alexa-488 labeled ant-Human IgG Fab plus either 33 nM CR3022 therapeutic antibody (αCR3022) (Yuan et al, 2020), B38 neutralizing antibody (αB38), or H4 neutralizing antibody (αH4) (Wu et al, 2020). Cells were washed with TBS and then analyzed in a V-bottom 96-well plate using a Guava EasyCyte HT flow cytometry system. For each condition, three replicates were measured with 3000 events collected per replicate.

3.2. P2 S Expression and Purification

To express SARS-CoV-2 P2 S encoded by BNT162b2 for biophysical characterization, a gene encoding the full length SARS-CoV-2 spike (GenBank: MN908947) with two prolines substituted at residues 986 and 987 (K986P and V987P) followed with a C-terminal HRV3C protease site and a TwinStrep tag was cloned into a modified pcDNA3.1(+) vector with the CAG promoter. The TwinStrep-tagged P2 S was expressed in Expi293F cells.

Purification of the recombinant protein was based on a procedure described previously, with minor modifications (Cai et al, 2020). Upon cell lysis, P2 S was solubilised in 1% NP-40 detergent. The TwinStrep-tagged protein was then captured with StrepTactin Sepharose HP resin in 0.5% NP-40. P2 S was further purified by size-exclusion chromatography and eluted as three distinct peaks in 0.02 % NP-40 as previously reported (Cai et al, 2020). 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.

3.3. Binding Kinetics of P2 S to Immobilized Human ACE2 and a Neutralizing Monoclonal Antibody by Biolayer Interferometry

Binding of NP-40 solubilized, purified P2 S to ACE2-PD and human neutralizing monoclonal antibody B38 (Wu et al, 2020) was measured by biolayer interferometry at 25 °C on an Octet RED384 (FortéBio). P2 S binding was measured in 25 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA and 0.02% NP-40. Avi-tagged human ACE2-PD was immobilized on streptavidin-coated sensors; B38 antibody was immobilized on protein G-coated sensors. For

a P2 S concentration series, after initial baseline equilibration of 120 seconds, the sensors were dipped in a 10 µg/mL solution of Avi-tagged ACE2-PD or B38 mAb for 300 seconds to achieve capture levels of 1 nM using the threshold function. Then, after another 120 seconds of baseline, binding data were collected for 300 seconds of association and 600 seconds of dissociation.

Biolayer interferometry data were collected with Octet Data Acquisition software version 10.0.0.87 and processed using FortéBio Data Analysis software version 10.0. Data were reference subtracted and fit to a 1:1 binding model with R^2 value greater than 0.95 to determine kinetics and affinity of binding using Octet Data Analysis Software v10.0 (FortéBio).

3.4. Cryo-EM of P2 S

For TwinStrep-tagged P2 S, 4 µL purified protein at 0.5 mg/mL were applied to gold Quantifoil R1.2/1.3 300 mesh grids freshly overlaid with graphene oxide. The sample was blotted using a Vitrobot Mark IV for 4 seconds with a force of -2 before being plunged into liquid ethane cooled by liquid nitrogen. 27,701 micrographs were collected from two identically prepared grids. Data were collected from each grid over a defocus range of -1.2 to -3.4 µm with a total electron dose of 50.32 and 50.12 $e^-/Å^2$, respectively, fractionated into 40 frames over a 6-second exposure for 1.26 and 1.25 $e^-/Å^2$/frame. On-the-fly motion correction, CTF estimation, and particle picking and extraction with a box size of 450 pixels were performed in Warp (Tegunov & Cramer, 2019), during which super-resolution data were binned to give a pixel size of 0.87 Å. A total of 1,119,906 particles were extracted. All subsequent processing was performed in RELION 3.1-beta (Zivanov et al, 2018). Particle heterogeneity was filtered out with 2D and 3D classification, yielding a set of 73,393 particles, which refined to 3.6 Å with C3 symmetry. 3D classification of this dataset without particle alignment separated out one class with a single RBD up, representing 15,098 particles. The remaining 58,295 particles, in the three RBD ‘down’ conformation, were refined to give a final model at 3.29 Å. The atomic model from PDB ID 6XR8 (Cai et al, 2020) was rigid-body fitted into the map density, then flexibly fitted to the density using real-space refinement in Phenix (Adams et al, 2010) alternating with manual building in Coot (Emsley et al, 2010). Data collection, 3D reconstruction and model refinement statistics are listed in Table 1.

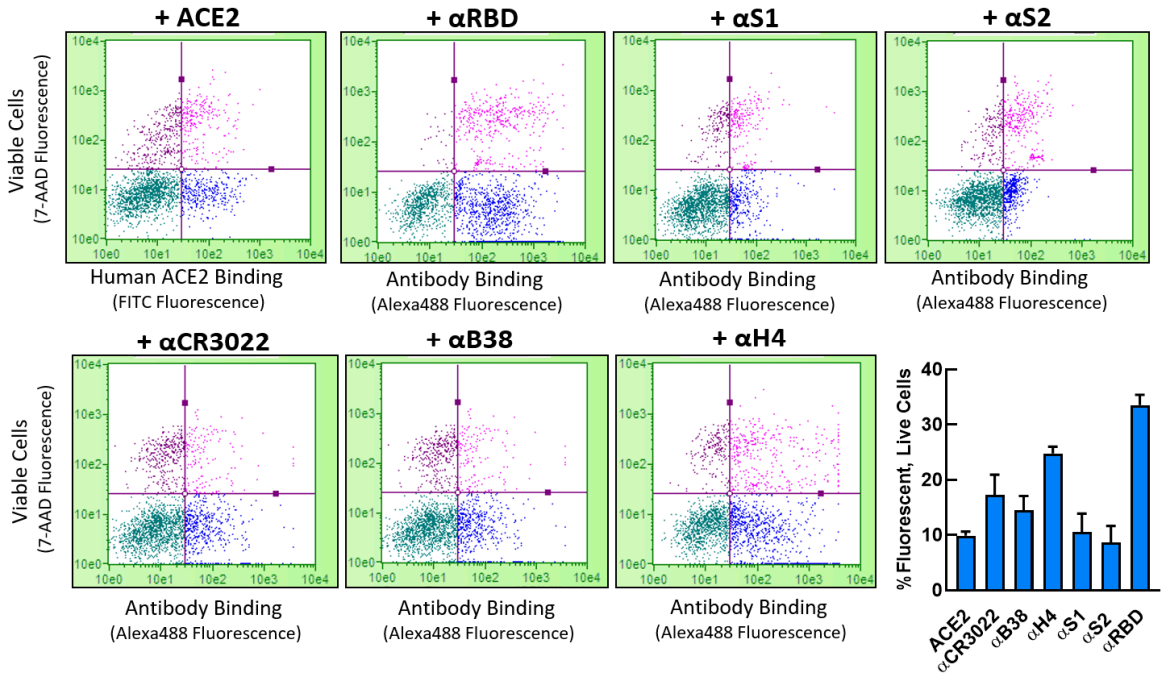
Table 1. CryoEM Data Collection, 3D Reconstruction and Refinement Statistics

Data Collection		
Electron microscopy equipment	Titan Krios (Thermo Fisher Scientific)	
Voltage (keV)	300	
Detector	K2 Summit	
Energy filter	Gatan GIF, 20 ev slit	
Nominal magnification	165,000 x	
Pixel size (Å)	0.435 (super-resolution)	
	Grid 1	**Grid 2**
Electron dose ($e^-/Å^2$)	50.32	50.12
Dose rate ($e^-/Å^2$/sec)	8.4	8.33
Defocus range (μm)	-1.2 to -3.4	-1.2 to -3.4
Number of collected micrographs	10,422	17,279
Number of selected micrographs	27,701	
3D Reconstruction		
Software	Warp, Relion	
Number of used particles	58,295	
Symmetry imposed	C3	
Global resolution (Å)		
Fourier shell correction = 0.143	3.29	
Applied B factor ($Å^2$)	-50	
Refinement		
Software	Phenix, Coot	
Protein residues	2,919	
Map correlation coefficient	0.82	
Root mean square deviation		
Bond length (Å)	0.011	
Bond angles (°)	0.962	
Ramachandran plot statistics (%):		
Preferred	90.4	
Allowed	9.59	
Outlier	0	
Poor rotamers (%)	11.06	
MolProbity score	2.96	
EMRinger score	2.23	
Clashscore (all atoms)	13.23	

4. RESULTS AND DISCUSSION

To confirm surface expression of untagged P2 S as well as the ability of P2 S to bind to human ACE2, flow cytometry experiments were performed on nonpermeabilized cells (Figure 1). Antibodies to the RBD, S1, and S2 were pre-incubated with Alexa-488 anti-IgG Fab for staining, and a nucleic acid dye was used to separate live and dead cells. To confirm binding of human ACE2, P2 S-expressing cells were labeled with the extracellular domain of human ACE2 pre-incubated with a FITC-labeled antibody against an affinity tag on the ACE2. Finally, anti-RBD human neutralizing antibodies B38 and H4 isolated from a COVID-19 convalescent patient (Wu et al, 2020) and the anti-RBD therapeutic antibody CR3022 (Yuan et al, 2020) were similarly confirmed to bind the surface-expressed P2 S.

Figure 1. Binding to Cell Surface-Expressed Recombinant P2 S

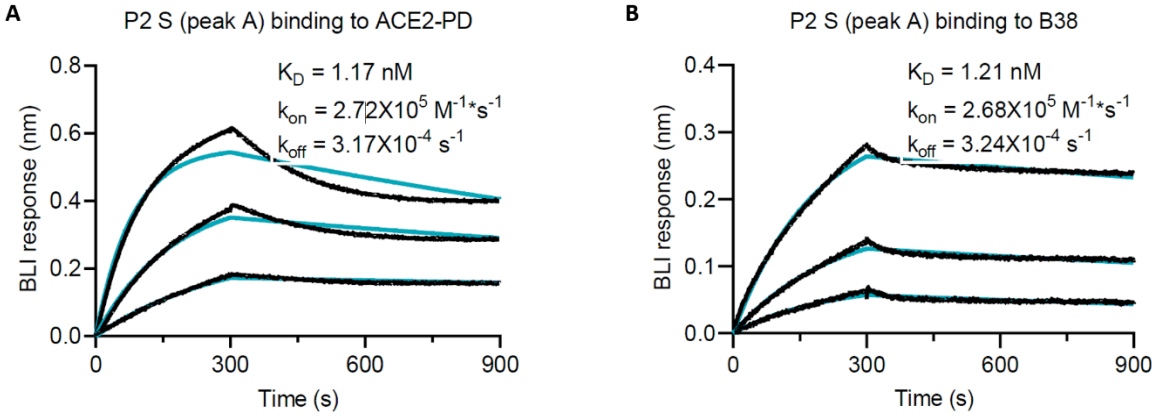


P2 S antigen was expressed in Expi293F cells, and surface expression confirmed by staining with antibodies against the RBD, S1, and S2 regions of the full-length S protein. Human ACE2 peptidase domain as well as the therapeutic antibody CR3022 and two neutralizing antibodies isolated from a COVID-19 convalescent patient, B38 and H4, were further confirmed to bind to surface express P2 S. The nucleic acid dye 7-AAD was used identify viable cells (lower quadrants in flow plots). Binding to surface expressed P2 S over background in live cells is quantified across replicates in the bar graph.

For structural and biophysical characterization, P2 S was expressed in Expi293F cells from DNA that encodes the same amino acid sequence as BNT162b2 RNA, with the addition of a C-terminal TwinStrep tag for affinity purification. Following purification, as described in Methods, P2 S eluted as three distinct peaks in 0.02% NP-40 as previously reported (Cai et al, 2020). Protein from the first peak of a size exclusion column, containing intact P2 S and dissociated S1 and S2, was assayed by biolayer interferometry (Figure 2). The trimeric

P2 S bound to the human ACE2-PD, and an anti-RBD human neutralizing antibody B38 with high affinity (apparent K_D = 1 nM).

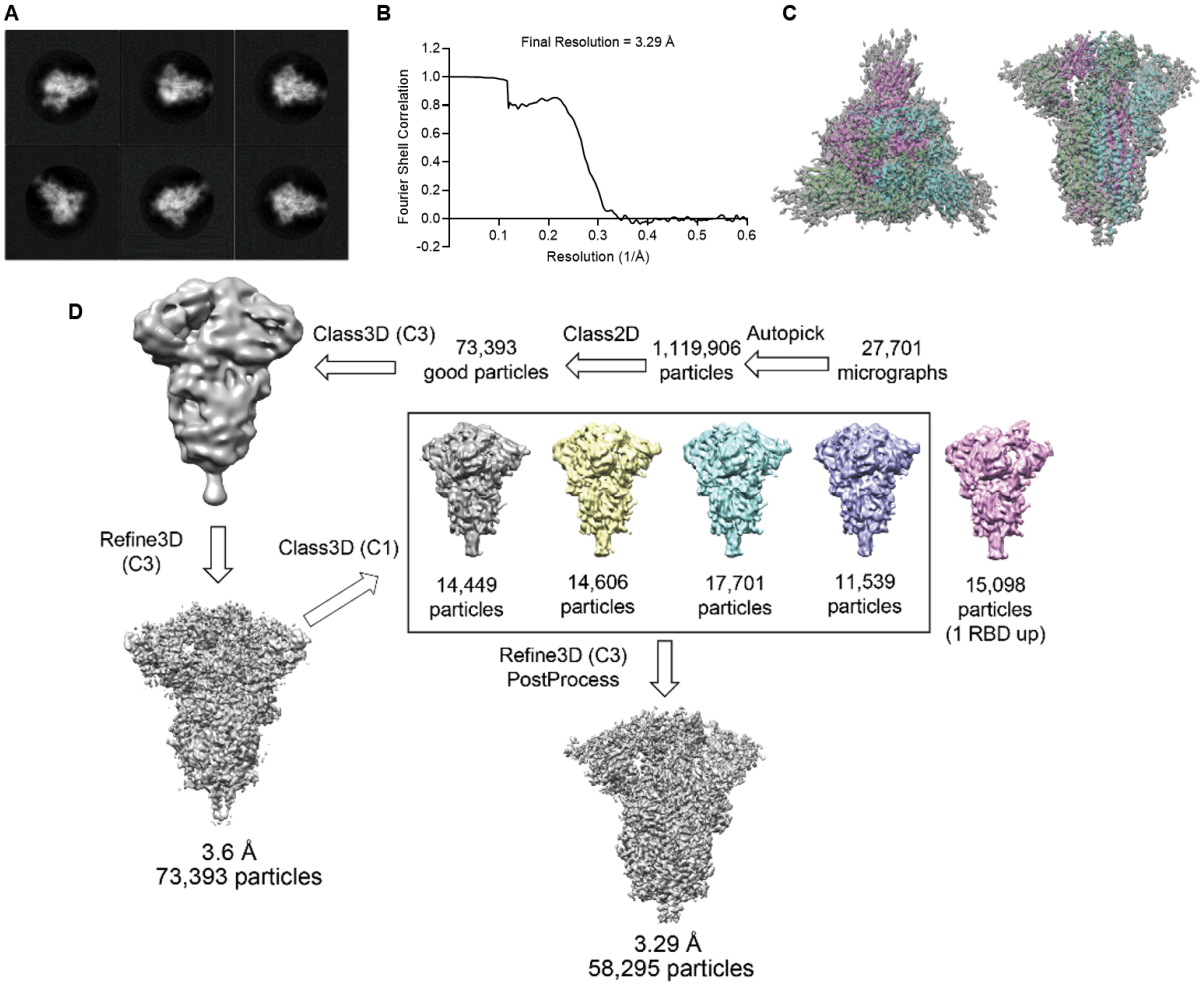
Figure 2. Biolayer Interferometry Sensorgrams for Binding of P2 S to ACE2-PD and B38 mAb



P2 S with a C-terminal TwinStrep tag expressed in Expi293F cells, was detergent solubilized and purified by affinity and size exclusion chromatography. Protein from the first peak of a size exclusion column, containing intact P2 S and dissociated S1 and S2, was assayed by biolayer interferometry on an Octet RED384 (FortéBio) at 25 °C in running buffer consisting of 25 mM Tris pH 7.5, 150 mM NaCl, 1mM EDTA and 0.02 % NP-40. Sensorgrams showing the binding kinetics of TwinStrep-tagged P2 S to immobilized **A**, human ACE2-PD and **B**, B38 monoclonal antibody. The highest concentration tested for P2 S was 71 nM with 2 more 3-fold dilutions. The binding curves were globally fit to a 1:1 Langmuir binding model with R^2 values greater than 0.95. Actual binding data (black) and the best fit of the data to a 1:1 binding model (green). Apparent kinetic parameters are provided in the graphs.

Purified TwinStrep-tagged P2 S was characterized structurally using cryoEM. 2D classification of particles from cryoEM data revealed a particle population that closely resembles the prefusion conformation of SARS-CoV-2 spike protein (Figure 3A). Processing and refinement of this dataset yielded a high-quality 3D map with a nominal resolution of 3.29 Å (Figure 3B), into which a previously published atomic model (PDB ID: 6VSB) was fitted and rebuilt. The rebuilt model (Figure 3C) shows good agreement with reported structures of prefusion full-length wild type S (Cai et al, 2020) and its ectodomain with P2 mutations (Wrapp et al, 2020). Three-dimensional classification of the dataset (Figure 3D) showed a class of particles that was in the one RBD ‘up’ (accessible for receptor binding), two RBD ‘down’ (closed) conformation and represented 20.4% of the trimeric molecules. The remainder were in the all RBD ‘down’ conformation. The RBD in the ‘up’ conformation was less well resolved than other parts of the structure, suggesting conformational flexibility and a dynamic equilibrium between RBD ‘up’ and RBD ‘down’ states as also suggested by others (Cai et al, 2020; Henderson et al, 2020).

Figure 3. CryoEM P2 S Structure at 3.29 Å Resolution



A. Representative 2D class averages of TwinStrep-tagged P2 S particles extracted from cryoEM micrographs. Box edge: 39.2 nm. **B.** Fourier shell correlation curve from RELION gold-standard refinement of the P2 S trimer. **C**. 3.29 Å cryoEM map of TwinStrep-tagged P2 S, with fitted atomic model, showing top (perpendicular to the three-fold axis) and side (parallel to the three-fold axis) views. CryoEM model is based on PDB 6VSB and was fitted into the structure using manual rebuilding in Coot and real-space refinement in Phenix. ~28,000 micrographs were collected using a Titan Krios electron microscope operating at 300 kV accelerating voltage, and image processing and 3D reconstructions were performed using Warp and RELION. **D**. Flowchart for cryo-EM data processing of the complex, showing 3D class averages. Maps of P2 S produced by 3D classification indicate some heterogeneity in positioning of the RBD domains. Percentages of the particle population represented in each class are indicated below the models.

5. CONCLUSION

We demonstrate that the BNT162b2 RNA sequence encodes a recombinant P2 S that can authentically present the ACE2 binding site and other epitopes targeted by SARS-CoV-2 neutralizing antibodies.

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 (Zost et al, 2020). The alternative states observed reflect a dynamic equilibrium between RBD 'up' and 'down' positions (Cai et al, 2020; Henderson et al, 2020). Binding of expressed and purified P2 S to ACE2 and a neutralizing monoclonal antibody further demonstrates its conformational and antigenic integrity.

6. DEVIATIONS

N/A

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A SINGLE DOSE PHARMACOKINETICS STUDY OF ALC-0315 AND ALC-0159 FOLLOWING INTRAVENOUS BOLUS INJECTION OF PF-07302048 NANOPARTICLE FORMULATION IN WISTAR HAN RATS

1. SUMMARY

Following a single IV bolus administration of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 (SARS-CoV-2 mRNA Vaccine; BioNTech code number BNT162) at 1 mg/kg to male Wistar Han rats, plasma concentrations of ALC-0315 and ALC-0159 (the novel excipients in PF-07302048) decreased rapidly, with initial $t_{½}$ of 1.62 and 1.72 h, respectively. ALC-0315 and ALC-0159 were then cleared from plasma, resulting in terminal elimination $t_{½}$ of 139 and 72.7 h, respectively.

The estimated percent of dose distributed to the liver was ~60% for ALC-0315 and ~20% for ALC-0159. The percent of dose excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. The percent of dose excreted unchanged in the urine was not calculated due to all values being BLQ.

2. OBJECTIVE

The PF-07302048 vaccine LNP formulation contains two novel excipients, ALC-0315 and ALC-0159, in the nanoparticle. The objective of this study is to assess the pharmacokinetics and elimination of ALC-0315 and ALC-0159 following a single IV bolus administration of a luciferase-encoding modRNA with an identical nanoparticle lipid composition as PF-07302048 at 1 mg/kg to male Wistar Han rats.

3. MATERIALS AND METHODS

3.1. Preparation of Doses

	IV
Study ID	PF-07302048_06Jul20_072424
Compound lot number	FM-1261-A
Dose of modRNA (mg/kg)	1 (1.96 mg/kg ALC-0159, 15.3 mg/kg ALC-0315)
Formulation concentration (mg/mL)	1
Dose volume (mL/kg)	1

3.2. Study Conduct

	IV
In-life location	Pfizer[a]
Species (strain)	Rat (Wistar Han)
Sex/number of animals	Male/3 animals per time point[b]
Feeding Condition	Fasted
Administration type	IV bolus
Administration site	Lateral tail vein
Sampling site	Inferior vena cava
Blood and liver sampling time points (h postdose)	Predose, 0.1, 0.25, 0.5, 1, 3, 6, 24, 48, 96, 192, 336
Anticoagulant	K_2EDTA
Urine and feces sample collection interval (h postdose)[c]	Predose (-24-0), 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240, 240-264, 264-288, 288-312, 312-336

a. Pfizer Worldwide Research, Development and Medical, San Diego, CA.
b. Non-serial sampling, 36 animals total.
c. Urine and feces were collected from animals 34-36, placed in metabolism cages.

3.3. Bioanalytical Summary for Quantitation of ALC-0315 and ALC-0159 in Plasma, Liver Homogenates, Urine, and Feces Homogenates

Bioanalytical Platform (instrument)	LC-MS/MS (AB Sciex QTRAP 5500)		
Mobile phase	A: 0.1% formic acid with 10 mM ammonium formate B: Acetonitrile with 10 mM ammonium formate and 0.1% formic acid		
Flow rate	0.25 mL/min		
Gradient	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.1	0	100
	0.5	0	100
	2.5	55	45
	4.2	55	45
	4.5	0	100
	6.5	0	100
Column	Waters Atlantis HILIC Silica 2.1 × 100 mm, 3µ		
Detection mode	Positive selected reaction monitoring mode		
Data collection software/ version	Analyst Version 1.7.1		
Data analysis software/ version	Watson Version 7.5		
MRM transitions	766.9→510.7 (ALC-0315) 839.2→494.7 (ALC-0159) 837.1→264.6 (PEG-2000, ISTD)		

Additional conditions can be found in the analysis files referenced in Section 5 (Archiving).

3.4. Method Summary

1. In brief, 20 µL aliquots of plasma, liver homogenate (sections from 3 areas in the liver were homogenized, pooled, and diluted 1:20 or 1:100 with blank sample matrix when necessary), urine, and feces homogenate (diluted 1:10 or 1:20 with blank sample matrix when necessary) samples and standards were subjected to protein precipitation with acetonitrile containing an internal standard, PEG-2000. Samples were vortexed and centrifuged to obtain supernatant, which was analyzed using LC-MS/MS.

2. Method information was recorded and archival records are available as described in Section 5 (Archiving).

3. Analyst® was used to measure peak areas and peak area ratios of analyte to internal standard were calculated. A calibration curve was constructed from the peak area ratios (analyte to internal standard) with a quadratic (1/x) regression using Watson LIMS. The linear dynamic ranges of the standard curves for ALC-0315 and ALC-0159 were 4.88 to 2500 ng/mL for plasma, 19.53 to 10000 ng/g for liver, 4.88 to 2500 ng/mL for urine, and 6.592 to 3375 ng/g for feces.

3.5. Data Analysis

Generation and analyses of pharmacokinetic data were conducted at Pfizer Inc. The pharmacokinetic parameters were determined from pooled animal data using non-compartmental analysis in Watson LIMS 7.5. For PK calculations, 0 µg/ml was used for C_0 for both ALC-0315 and ALC-0159. For pharmacokinetic definitions and calculations, see Appendix 8.1. BLQ concentrations were not used in the PK calculations.

4. RESULTS AND DISCUSSION

Mean pharmacokinetic parameters of ALC-0315 and ALC-0159 following administration of a single IV dose of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 to male Wistar Han rats at 1 mg/kg are shown in Supportive Table 6.1. Mean concentration-time data of ALC-0315 and ALC-0159 from plasma, liver homogenates, urine, and feces are shown in Supportive Tables 6.2, 6.3, 6.5, and 6.6. The ratio of plasma concentrations of ALC-0315:ALC-0159 is shown in Supportive Table 6.4. The concentration-time profiles of plasma, liver, and feces following IV administration of the LNP are shown in Supportive Figures 7.1, 7.2, and 7.3, respectively.

Following a single IV dose of a luciferase-encoding modRNA formulated in an LNP with an identical lipid composition as PF-07302048 at 1 mg/kg to Wistar Han rats, plasma concentrations of ALC-0315 and ALC-0159 decreased rapidly, with initial $t_{½}$ values of 1.62 and 1.72 h, respectively. ALC-0315 and ALC-0159 were then cleared from plasma, resulting in terminal elimination $t_{½}$ of 139 and 72.7 h, respectively.

The estimated percent of dose distributed to the liver was ~60% for ALC-0315 and ~20% for ALC-0159. The percent of dose excreted unchanged in feces was ~1% for ALC-0315 and ~50% for ALC-0159. The percent of dose excreted unchanged in the urine was not calculated due to values being BLQ.

5. ARCHIVING

Data presented in this report can be found in the following locations:

	Experimental Data
E-Workbook	/Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200801 PF-07302048_06Jul20_072424_PLM /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200805 PF-07302048_06Jul20_072424_liver /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200806 PF-07302028_06Jul20_072424_Urine /Root/PDM/ (b) (6) VBN#00701419/VR_LNP/20200809 PF-07302048_06Jul20_072424_Feces
Watson LIMS	PROJECT ID: PF-07302048 STUDY ID: Covidvac_072414PK

	Bioanalytical Data
E-Workbook	/Root/PDM/ (b) (6) VBN#00701419/VR_LNP/Assay Development/LC-MSMS method for COVID-19 Excipients
OpenLAB LAJ_PDM	\COMPOUND\PF-07302048\Covidvac_072424PK\PLM.wiff

6. SUPPORTIVE TABLES

6.1. Summary of Mean Plasma Pharmacokinetic Parameters of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Species (Strain)	Rat (Wistar Han)	
Sex/Number of Animals	Male/ 3 animals per timepoint[a]	
Feeding Condition	Fasted	
Method of Administration	IV	
Dose modRNA (mg/kg)	1	
Dose ALC-0159 (mg/kg)	1.96	
Dose ALC-0315 (mg/kg)	15.3	
Sample Matrix	Plasma	
Sampling Time Points (h post dose):	Predose, 0.1, 0.25, 0.5, 1, 3, 6, 24, 48, 96, 192, 336	
Analyte	ALC-0315	ALC-0159
PK Parameters:	Mean[b]	Mean[b]
AUC_{inf} (µg•h/mL)[c]	1030	99.2
AUC_{last} (µg•h/mL)	1020	98.6
Initial $t_{½}$ (h)[d]	1.62	1.74
Terminal elimination $t_{½}$ (h)[e]	139	72.7
Estimated fraction of dose distributed to liver (%)[f]	59.5	20.3
Dose in Urine (%)	NC[g]	NC[g]
Dose in Feces (%)	1.05	47.2

a. Non-serial sampling, 36 animals total.
b. Only mean PK parameters are reported due to non-serial sampling.
c. Calculated using the terminal log-linear phase (determined using 48, 96, 192, and 336 h for regression calculation).
d. ln(2)/initial elimination rate constant (determined using 1,3, and 6 h for regression calculation).
e. ln(2)/terminal elimination rate constant (determined using 48, 96, 192, and 336 for regression calculation).
f. Calculated as follows: highest mean amount in the liver (µg)/total mean dose (µg) of ALC-0315 or ALC-0159.
g. Not calculated due to BLQ data.

6.2. Summary of Mean and Individual Plasma and Liver Concentrations of ALC-0315 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Animal Number	Animal weight (g)	Time (h postdose)	Plasma (µg/mL)	Liver (µg/g)	Liver weight (g)	Total amount in liver (µg)
1	--	Predose	BLQ	BLQ	5.78	BLQ
2	--	Predose	BLQ	BLQ	5.57	BLQ
3	--	Predose	BLQ	BLQ	5.89	BLQ
Mean ± SD	**--**	**Predose**	**BLQ ± NA**	**BLQ ± NA**	**--**	**BLQ ± NA**
4	221	0.1	456	28.5	5.74	163
5	225	0.1	564	30.2	5.62	170
6	220	0.1	435	30.9	5.52	171
Mean ± SD	**--**	**0.1**	**485 ± 69.2**	**29.9 ± 1.23**	**--**	**168 ± 3.93**
7	222	0.25	418	74.1	5.94	440
8	227	0.25	451	90.6	5.91	536
9	218	0.25	191	29.0	5.54	161
Mean ± SD	**--**	**0.25**	**353 ± 142**	**64.6 ± 31.9**	**--**	**379 ± 195**
10	228	0.5	356	164	6.49	1060
11	217	0.5	333	143	5.87	839
12	224	0.5	423	140	5.03	705
Mean ± SD	**--**	**0.5**	**371 ± 46.8**	**149 ± 13.1**	**--**	**869 ± 182**
13	212	1	204	228	6.04	1380
14	221	1	208	228	6.29	1430
15	238	1	172	240	6.40	1540
Mean ± SD	**--**	**1**	**195 ± 19.7**	**232 ± 6.93**	**--**	**1450 ± 80.3**
16	220	3	85.3	282	6.45	1820
17	209	3	71.2	318	5.63	1790
18	210	3	83.1	282	5.62	1580
Mean ± SD	**--**	**3**	**79.9 ± 7.59**	**294 ± 20.8**	**--**	**1730 ± 129**
19	222	6	24.2	270	6.04	1630
20	222	6	20.2	279	5.50	1530
21	222	6	24.4	256	6.56	1680
Mean ± SD	**--**	**6**	**22.9 ± 2.37**	**268 ± 11.6**	**--**	**1620 ± 74.4**
22	228	24	1.13	277	7.26	2010
23	229	24	1.11	281	7.48	2100
24	231	24	0.861	285	7.52	2140
Mean ± SD	**--**	**24**	**1.03 ± 0.150**	**281 ± 4.00**	**--**	**2090 ± 68.4**
25	222	48	0.253	218	7.59	1650
26	228	48	0.339	206	7.90	1630
27	223	48	0.399	166	7.39	1230
Mean ± SD	**--**	**48**	**0.330 ± 0.0734**	**197 ± 27.2**	**--**	**1500 ± 240**
28	216	96	0.188	187	7.84	1470
29	224	96	0.122	114	8.81	1000
30	242	96	0.192	97.1	9.86	957
Mean ± SD	**--**	**96**	**0.167 ± 0.0393**	**133 ± 47.8**	**--**	**1140 ± 282**
31	216	192	0.0812	66.9	9.10	609
32	212	192	0.135	60.7	8.34	506
33	233	192	0.110	90.3	9.59	866
Mean ± SD	**--**	**192**	**0.109 ± 0.0269**	**72.6 ± 15.6**	**--**	**660 ± 185**
34	210	336	0.0538	53.6	10.1	541
35	213	336	0.0724	95.3	8.13	774
36	219	336	0.0801	62.2	11.0	682
Mean ± SD	**--**	**336**	**0.0688 ± 0.0135**	**70.4 ± 22.0**	**--**	**666 ± 118**

The limit of quantitation was 0.00488 µg/mL for plasma and 0.01953 µg/g for liver.

6.3. Summary of Mean and Individual Plasma and Liver Concentrations of ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Animal Number	Animal weight (g)	Time (h postdose)	Plasma (µg/mL)	Liver (µg/g)	Liver weight (g)	Total amount in liver (µg)
1	--	Predose	BLQ	BLQ	5.78	BLQ
2	--	Predose	BLQ	BLQ	5.57	BLQ
3	--	Predose	BLQ	BLQ	5.89	BLQ
Mean ± SD	**--**	**Predose**	**BLQ ± NA**	**BLQ ± NA**	**--**	**BLQ ± NA**
4	221	0.1	48.8	7.07	5.74	40.6
5	225	0.1	57.9	8.03	5.62	45.1
6	220	0.1	43.9	7.65	5.52	42.3
Mean ± SD	**--**	**0.1**	**50.2 ± 7.10**	**7.58 ± 0.483**	**--**	**42.7 ± 2.32**
7	222	0.25	37.9	10.8	5.94	64.1
8	227	0.25	41.9	12.5	5.91	73.9
9	218	0.25	18.6	4.48	5.54	24.8
Mean ± SD	**--**	**0.25**	**32.8 ± 12.5**	**9.26 ± 4.23**	**--**	**54.3 ± 26.0**
10	228	0.5	33.8	15.0	6.49	97.3
11	217	0.5	28.3	14.4	5.87	84.5
12	224	0.5	29.0	16.2	5.03	81.5
Mean ± SD	**--**	**0.5**	**30.4 ± 2.99**	**15.2 ± 0.917**	**--**	**87.8 ±8.40**
13	212	1	14.1	15.2	6.04	91.8
14	221	1	18.5	13.3	6.29	83.6
15	238	1	15.2	14.3	6.40	91.5
Mean ± SD	**--**	**1**	**15.9 ± 2.29**	**14.3 ± 0.950**	**--**	**89.0 ± 4.63**
16	220	3	7.67	12.6	6.45	81.3
17	209	3	5.55	12.9	5.63	72.6
18	210	3	6.64	13.6	5.62	76.4
Mean ± SD	**--**	**3**	**6.62 ± 1.06**	**13.0 ± 0.513**	**--**	**76.8 ± 4.35**
19	222	6	1.94	7.74	6.04	46.8
20	222	6	1.98	7.12	5.50	39.1
21	222	6	2.50	7.80	6.56	51.2
Mean ± SD	**--**	**6**	**2.14 ± 0.312**	**7.55 ± 0.376**	**--**	**45.7 ± 6.09**
22	228	24	0.270	2.14	7.26	15.5
23	229	24	0.251	1.58	7.48	11.8
24	231	24	0.223	1.64	7.52	12.3
Mean ± SD	**--**	**24**	**0.248 ± 0.0236**	**1.79 ± 0.307**	**--**	**13.2 ± 2.01**
25	222	48	0.113	0.565	7.59	4.29
26	228	48	0.105	0.593	7.90	4.69
27	223	48	0.0842	0.546	7.39	4.03
Mean ± SD	**--**	**48**	**0.101 ± 0.0149**	**0.568 ± 0.0236**		**4.34 ± 0.329**
28	216	96	0.0631	0.216	7.84	1.69
29	224	96	0.0385	0.138	8.81	1.22
30	242	96	0.0524	0.148	9.86	1.46
Mean ± SD	**--**	**96**	**0.0513 ± 0.0123**	**0.167 ± 0.0424**	**--**	**1.46 ± 0.239**
31	216	192	0.0182	0.0647	9.10	0.589
32	212	192	0.0204	0.0553	8.34	0.461
33	233	192	0.0226	0.0619	9.59	0.593
Mean ± SD	**--**	**192**	**0.0204 ± 0.00220**	**0.0606 ± 0.00483**	**--**	**0.548 ± 0.0750**
34	210	336	0.00568	BLQ	10.1	BLQ
35	213	336	0.00619	BLQ	8.13	BLQ
36	219	336	0.00639	BLQ	11.0	BLQ
Mean ± SD	**--**	**336**	**0.00609 ± 0.000366**	**BLQ ± NA**	**--**	**BLQ ± NA**

The limit of quantitation was 0.00488 µg/mL for plasma and 0.01953 µg/g for liver.

6.4. Summary of the Ratio of ALC-0315:ALC-0159 in Plasma of Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

Time postdose (h)	Mean plasma concentration ALC-0315 (µg/mL)	Mean plasma concentration ALC-0159 (µg/mL)	Ratio (ALC-0315)/(ALC-0159)[a]
0	BLQ	BLQ	NA
0.1	485	50.2	9.66
0.25	353	32.8	10.8
0.5	371	30.4	12.2
1	195	15.9	12.3
3	79.9	6.62	12.1
6	22.9	2.14	10.7
24	1.03	0.248	4.15
48	0.330	0.101	3.27
96	0.167	0.0513	3.26
192	0.109	0.0204	5.34
336	0.0688	0.00609	11.3

The limit of quantitation was 0.00488 µg/mL.
a. Ratio prior to injection is 7.8 (15.3 mg/kg/1.96 mg/kg)

6.5. Summary of Mean and Individual Urine Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

	ALC-0315				ALC-0159			
	Individual Animal			Mean ± SD	Individual Animal			Mean ± SD
	Animal No. 34	Animal No. 35	Animal No. 36		Animal No. 34	Animal No. 35	Animal No. 36	
Animal Weight (g)	210	213	219	--	210	213	219	--
Time (h postdose)	Urine Concentration (ng/mL)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
24-48	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
48-72	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
72-96	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
96-120	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
120-144	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
144-168	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
168-192	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
192-216	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
216-240	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
240-264	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
264-288	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
288-312	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
312-336	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
Time (h)	Urine Volume (mL)							
Predose	22.0[a]	17.0	15.0[a]	--	22.0[a]	17.0	15.0[a]	--
0-24	11.0	10.0	15.0	--	11.0	10.0	15.0	--
24-48	9.00	7.50	12.0	--	9.00	7.50	12.0	--
48-72	10.0	10.0	10.0	--	10.0	10.0	10.0	--
72-96	12.0	10.0	9.00	--	12.0	10.0	9.00	--
96-120	15.0	12.0	12.5	--	15.0	12.0	12.5	--
120-144	14.5	11.5	12.5	--	14.5	11.5	12.5	--
144-168	13.0	9.00	10.0	--	13.0	9.00	10.0	--
168-192	15.0[b]	12.5	14.5	--	15.0[b]	12.5	14.5	--
192-216	13.0	8.00	12.5	--	13.0	8.00	12.5	--
216-240	12.0	8.00	12.0	--	12.0	8.00	12.0	--
240-264	15.0	14.0	16.0	--	15.0	14.0	16.0	--
264-288	9.50	8.00	12.0	--	9.50	8.00	12.0	--
288-312	13.0	10.0	15.0	--	13.0	10.0	15.0	--
312-336	16.0	10.0	15.0	--	16.0	10.0	15.0	--

The limit of quantitation was 0.00488 µg/mL
a. Possible water contamination.
b. Urine overflowed.

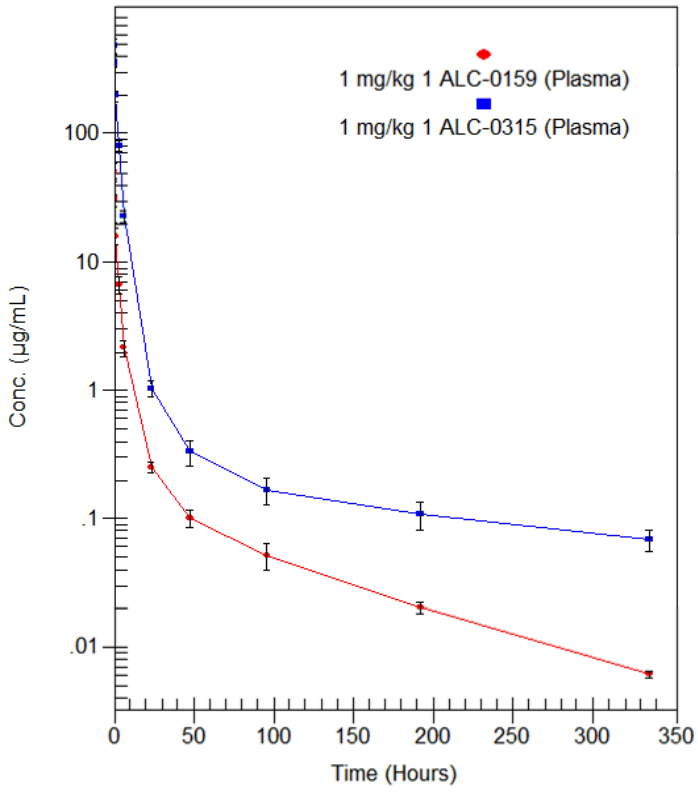
6.6. Summary of Mean and Individual Fecal Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg

	ALC-0315				ALC-0159			
	Individual Animal			Mean ± SD	Individual Animal			Mean ± SD
	No. 34	No. 35	No. 36		No. 34	No. 35	No. 36	
Animal Weight (g)	210	213	219	--	210	213	219	--
Time (h postdose)	Fecal Concentration (µg/g)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	1.50	0.474	0.397	0.790 ± 0.616	38.9	28.0	15.2	27.4 ± 11.9
24-48	0.972	0.432	0.778	0.727 ± 0.274	2.17	5.64	4.79	4.20 ±1.81
48-72	0.355	0.572	1.33	0.752 ± 0.512	0.710	0.952	1.33	0.997 ± 0.312
72-96	0.167	0.294	0.450	0.304 ± 0.142	0.401	0.389	0.421	0.404 ± 0.0162
96-120	0.369	0.114	0.173	0.219 ± 0.133	0.332	0.269	0.217	0.273 ± 0.0576
120-144	0.239	0.0881	0.113	0.147 ± 0.0809	0.375	0.171	0.143	0.230 ± 0.127
144-168	0.0888	0.100	0.175	0.121 ± 0.0469	0.300	0.157	0.228	0.228 ± 0.0715
168-192	0.110	0.0783	0.129	0.106 ± 0.0256	0.145	0.124	0.147	0.139 ± 0.0127
192-216	0.0790	0.0809	0.183	0.114 ± 0.0595	0.0904	0.163	0.146	0.133 ± 0.0380
216-240	0.142	0.152	0.101	0.132 ± 0.0270	0.155	0.182	0.116	0.151 ± 0.0332
240-264	0.0781	0.0764	0.135	0.0965 ± 0.0334	0.109	0.0783	0.142	0.110 ± 0.0319
264-288	0.0947	0.0635	0.122	0.0934 ± 0.0293	0.0754	0.0641	0.109	0.0828 ± 0.0234
288-312	0.0457	0.0758	0.107	0.0762 ± 0.0307	0.106	0.0580	0.0982	0.0874 ± 0.0258
312-336	0.0290	0.0641	0.0993	0.0641 ± 0.0352	0.0605	0.0494	0.0854	0.0651 ± 0.0184
Time (h)	Feces weight (g)							
Predose	4.80	4.40	6.40	--	4.80	4.40	6.40	--
0-24	2.50	7.90	5.50	--	2.50	7.90	5.50	--
24-48	8.90	5.50	8.70	--	8.90	5.50	8.70	--
48-72	11.5	8.60	13.1	--	11.5	8.60	13.1	--
72-96	11.8	13.1	10.5	--	11.8	13.1	10.5	--
96-120	13.9	10.6	13.2	--	13.9	10.6	13.2	--
120-144	10.9	12.9	12.6	--	10.9	12.9	12.6	--
144-168	13.5	15.3	8.20	--	13.5	15.3	8.20	--
168-192	8.40	15.4	10.5	--	8.40	15.4	10.5	--
192-216	13.0	11.3	8.00	--	13.0	11.3	8.00	--
216-240	10.1	11.1	9.80	--	10.1	11.1	9.80	--
240-264	10.4	11.4	9.40	--	10.4	11.4	9.40	--
264-288	11.1	11.2	8.40	--	11.1	11.2	8.40	--
288-312	11.7	11.6	9.70	--	11.7	11.6	9.70	--
312-336	12.0	11.5	7.20	--	12.0	11.5	7.20	--
Time (h)	Amount excreted in feces (µg)							
Predose	BLQ	BLQ	BLQ	BLQ ± NA	BLQ	BLQ	BLQ	BLQ ± NA
0-24	3.75	3.74	2.18	3.23 ± 0.903	97.3	221	83.6	134± 75.8
24-48	8.65	2.38	6.77	5.93 ± 3.22	19.3	31.0	41.7	30.7 ± 11.2
48-72	4.08	4.92	17.4	8.81 ± 7.47	8.17	8.19	17.4	11.3 ± 5.34
72-96	1.97	3.85	4.73	3.52 ± 1.41	4.73	5.10	4.42	4.75 ± 0.338
96-120	5.13	1.21	2.28	2.87 ± 2.03	4.61	2.85	2.86	3.44 ± 1.01
120-144	2.61	1.14	1.42	1.72 ± 0.778	4.09	2.21	1.80	2.70 ± 1.22
144-168	1.20	1.53	1.44	1.39 ± 0.171	4.05	2.40	1.87	2.77 ± 1.14
168-192	0.924	1.21	1.35	1.16 ± 0.219	1.22	1.91	1.54	1.56 ± 0.346
192-216	1.03	0.914	1.46	1.14 ± 0.290	1.18	1.84	1.17	1.40 ± 0.387
216-240	1.43	1.69	0.990	1.37 ± 0.353	1.57	2.02	1.14	1.57 ± 0.442
240-264	0.812	0.871	1.27	0.984 ± 0.249	1.13	0.893	1.33	1.12 ± 0.221
264-288	1.05	0.711	1.02	0.929 ± 0.189	0.837	0.718	0.916	0.823 ±0.100
288-312	0.535	0.879	1.04	0.817 ± 0.257	1.24	0.673	0.953	0.955 ± 0.284
312-336	0.348	0.737	0.715	0.600 ± 0.219	0.726	0.568	0.615	0.636 ± 0.0811

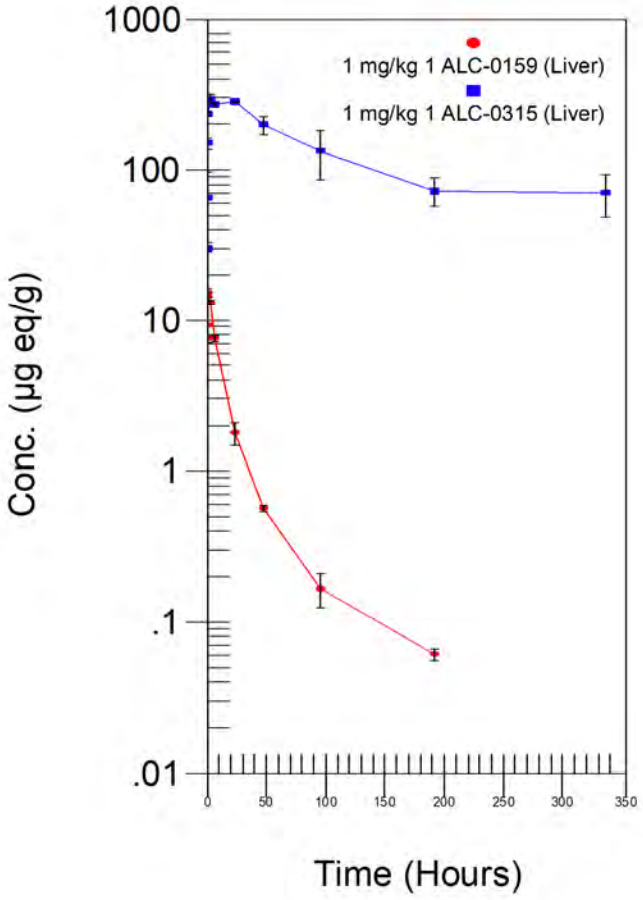
The limit of quantitation was 0.006592 µg/mL.

7. SUPPORTIVE FIGURES

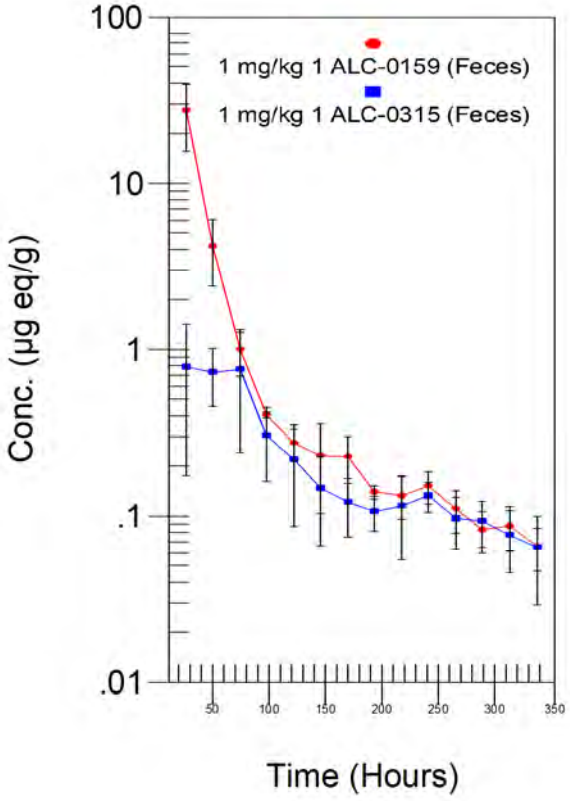
7.1. Mean Plasma Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



7.2. Mean Liver Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



7.3. Mean Fecal Concentrations of ALC-0315 and ALC-0159 in Male Wistar Han Rats Following Single IV Administration of Luciferase-encoding modRNA Formulated in an LNP with an Identical Lipid Composition as PF-07302048 at 1 mg/kg



8. SUPPORTIVE APPENDIX

8.1. Abbreviations and Pharmacokinetic Calculations

--	Data not available
AUC_{inf}	Area under the plasma drug concentration-time curve from 0 to infinite time. AUC_t plus extrapolated area determined by dividing plasma concentration at t by the slope of the terminal log-linear phase.
AUC_{last}	Area under the plasma drug concentration-time curve from 0 to the last quantifiable time point. Determined using the linear trapezoidal method.
BLQ	Below the limit of quantitation
K_2EDTA	Potassium ethylene diamine tetraacetic acid
ID	Identification
Initial $t_{½}$	Half-life. ln(2)/initial elimination rate constant
ISTD	Internal standard
IV	Intravenous
LNP	Lipid nanoparticle
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MRM	Multiple reaction monitoring
NA	Not applicable
NC	Not calculated
PEG	Polyethylene glycol
PK	Pharmacokinetic
SD	Standard deviation
Terminal elimination $t_{½}$	Half-life. ln(2)/terminal elimination rate constant
Dose in Feces (%)	Fecal excretion. (Mean µg of analyte in feces/ Mean µg of analyte administered) × 100
Dose in Urine (%)	Urinary excretion. (Mean µg of analyte in urine/ Mean µg of analyte administered) × 100

9. OTHER CONTRIBUTING SCIENTISTS

The following scientists were involved in the conduct of the PK portion of this study and are responsible for the scientific content of this research report.

ADME Associate/
Portfolio Support Group
Scientist

Bioanalytical Associate

(b) (6)

10. CHANGE HISTORY

Version	Sections	Revisions
1.0	NA	New document
2.0	Summary, Section 3.1, Results and Discussion, Supportive Table 6.1	Updated the doses of ALC-0159 and ALC-0315 and the percent of dose distributed to the liver and excreted in feces.

11. APPROVAL SIGNATURES

The author and approver are responsible for providing a true representation of the data.

(b) (6)
Report Author
Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, USA

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Report Approver
Pharmacokinetics, Dynamics and Metabolism, Pfizer, San Diego, CA, USA

Document Approval Record

Document Name:	PF-07302048_06Jul20_072424_A Single Dose Pharmacokinetics Study of ALC-0315 and ALC-0159 Following Intravenous Bolus Injection of PF-07302048 Nanoparticle Formulation in Wistar Han Rats
Document Title:	PF-07302048_06Jul20_072424_A Single Dose Pharmacokinetics Study of ALC-0315 and ALC-0159 Following Intravenous Bolus Injection of PF-07302048 Nanoparticle Formulation in Wistar Han Rats

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	11-Sep-2020 12:52:54	Author Approval
	11-Sep-2020 15:59:06	Final Approval

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.

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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)
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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

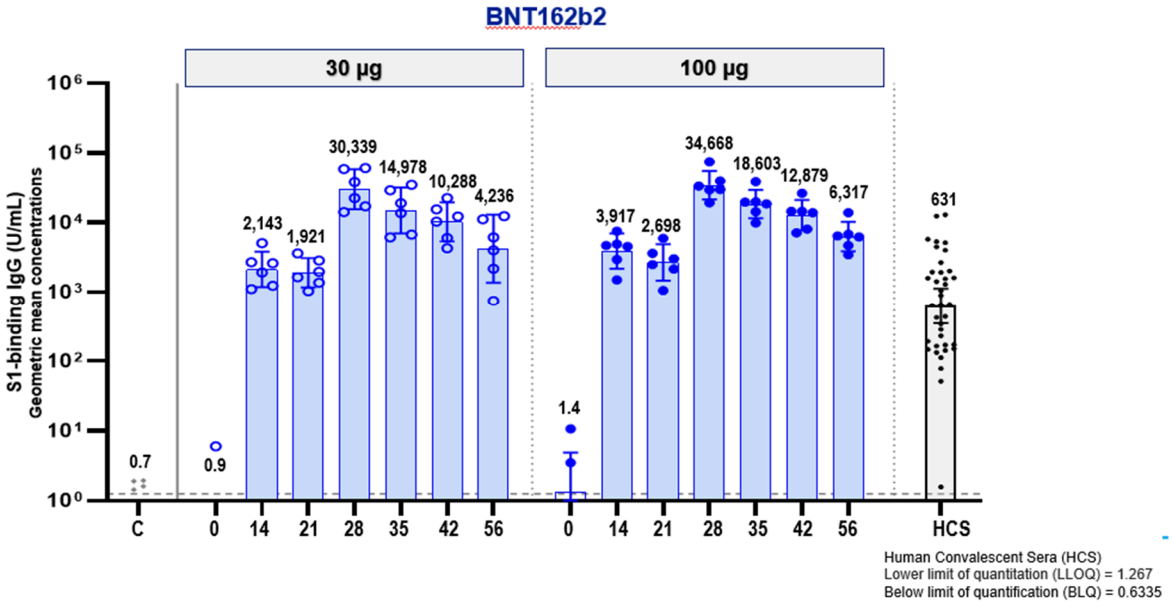
Group	Number of Animals (Animal ID)
Control[a]	3 (A16N193, A17N037, A17N102)
BNT162b2	6 (A17N109, A17N139, A17N167, A17N105, A17N113, A17N114)

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.

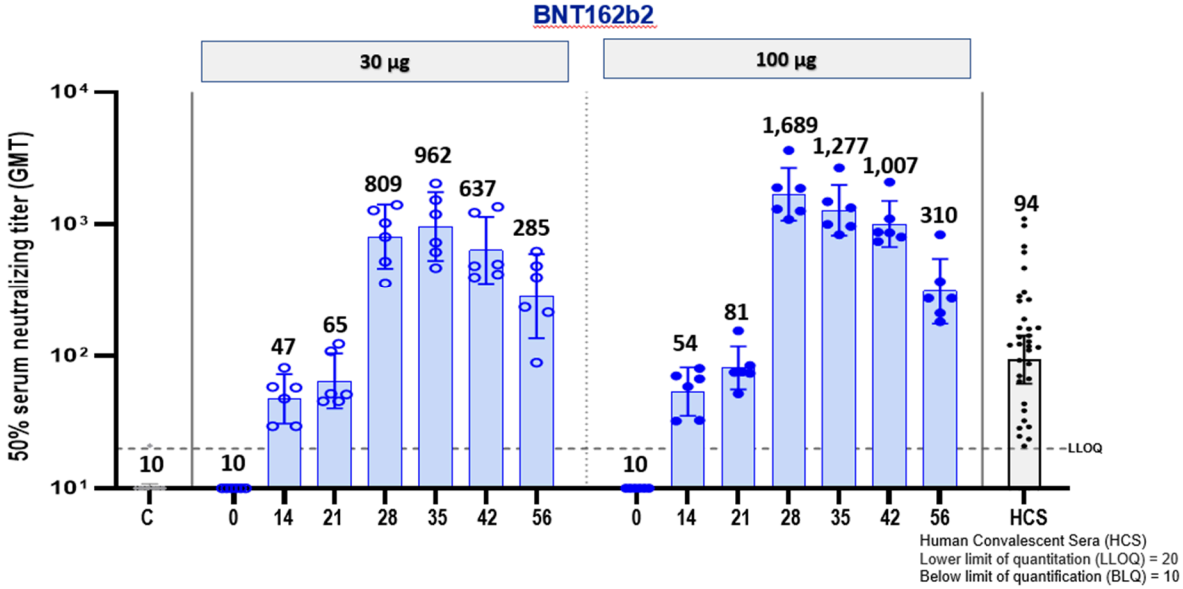
Figure 1. S1-binding IgG Concentrations Elicited by Immunization of Rhesus Macaques with BNT162b2 (V9)



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.

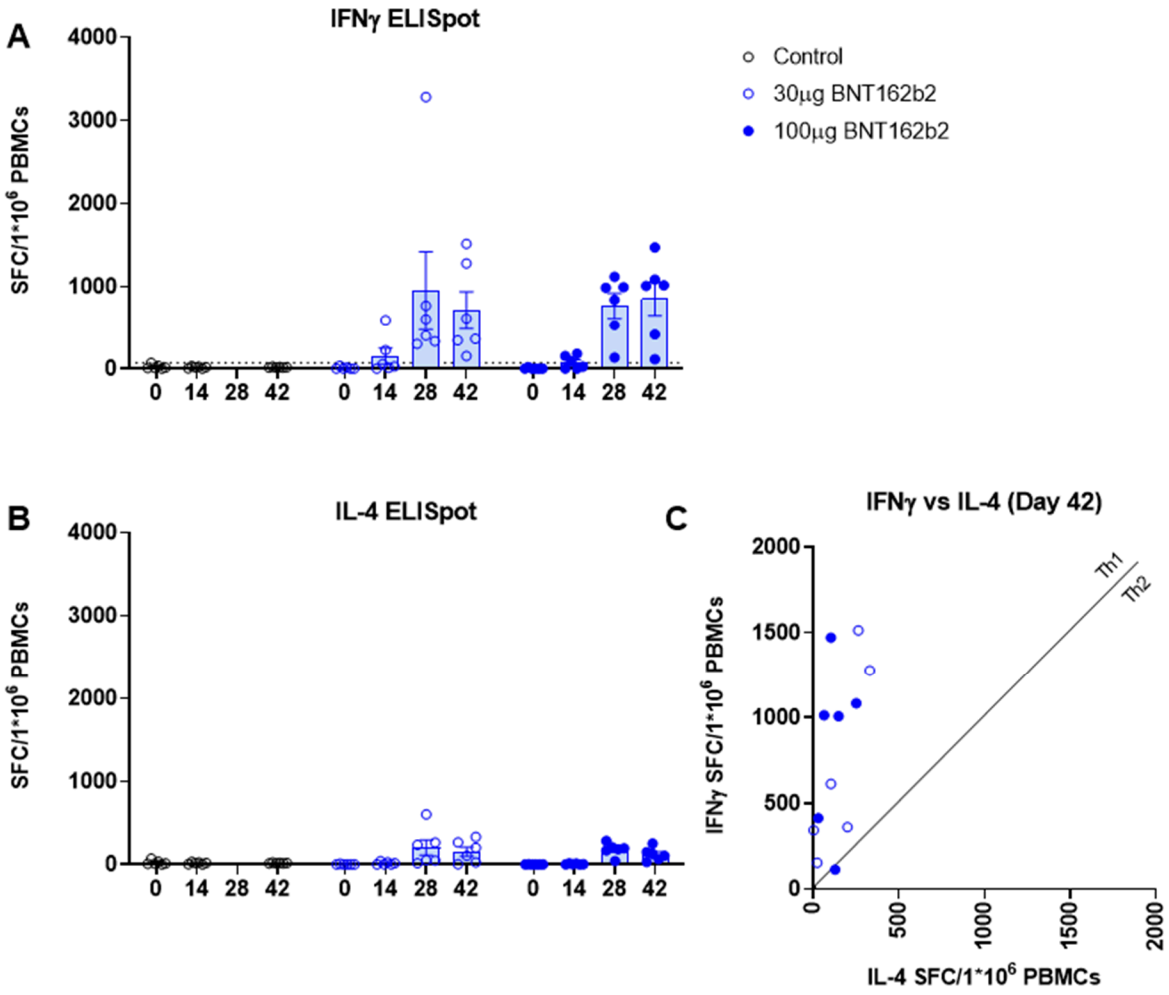
Figure 2. 50% Serum Neutralizing Titers Elicited by Immunization of Rhesus Macaques with BNT162b2 (V9)



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.

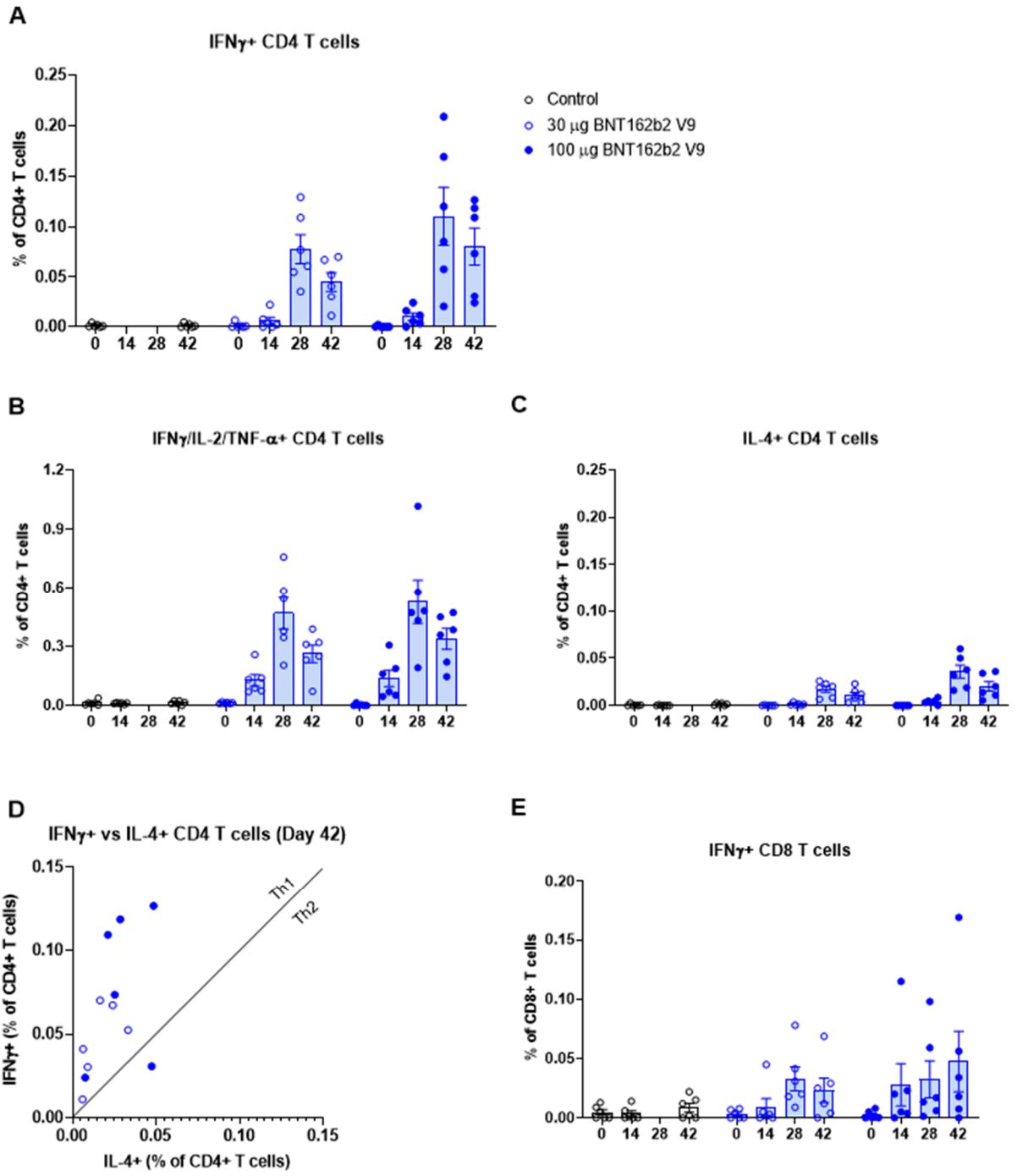
S-specific T-cell responses were analyzed by ELISpot and intracellular cytokine staining (ICS). Peripheral blood mononuclear cells (PBMCs) were collected before immunization and at the times indicated after Doses 1 and 2. In BNT162b2-immunized animals, strong IFNγ but minimal IL-4 responses were detected 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



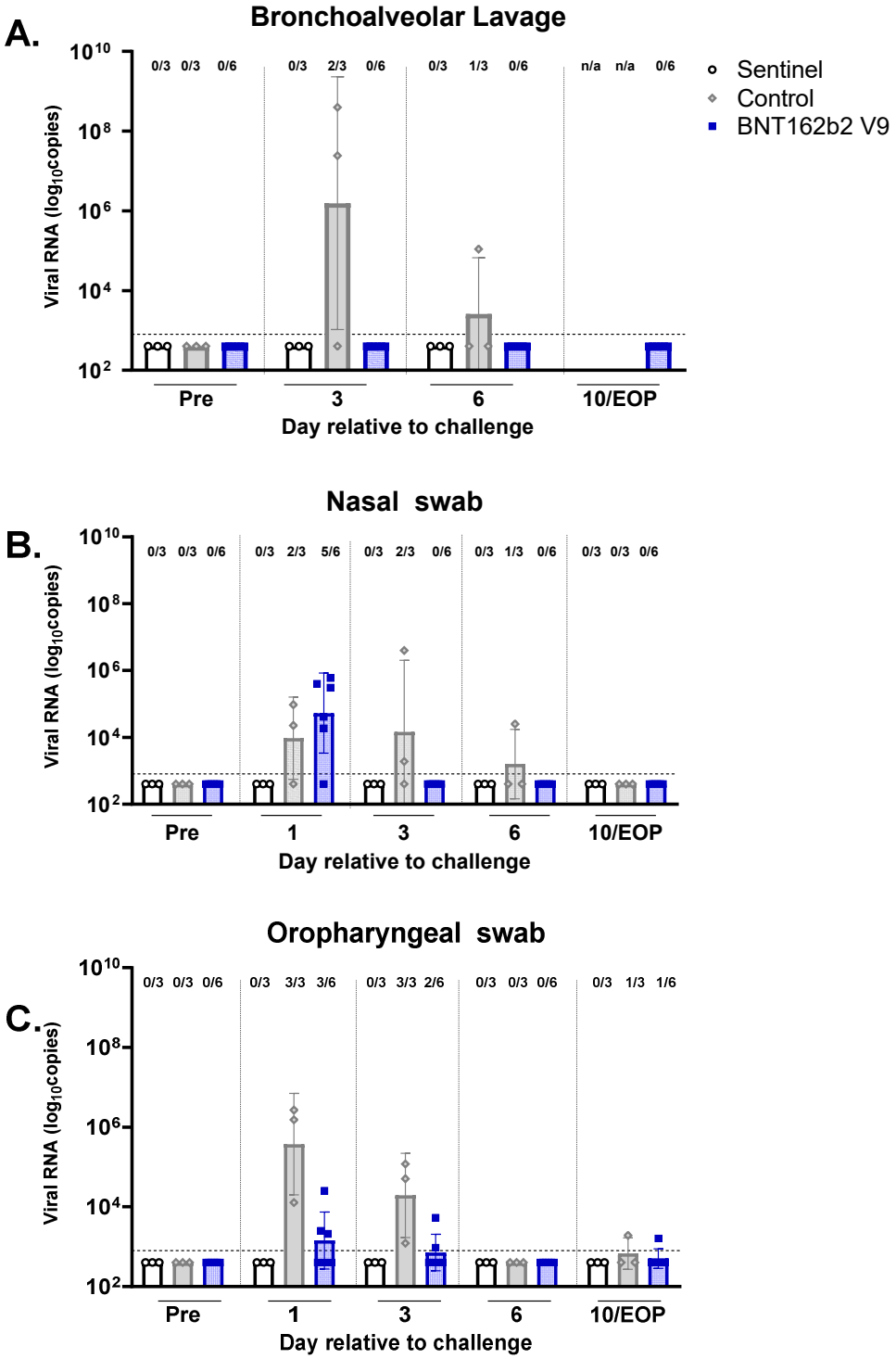
Numbers on the x-axis indicate the day post first immunization. Height of bars indicates the mean, whiskers indicate the standard error of mean (SEM), every symbol represents one animal. (A) Frequency of IFNγ+ CD4 T cells. (B) Frequency of IFNγ/IL-2/TNF-α+ CD4 T cells (C) Frequency of IL-4+ CD4 T cells. (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).

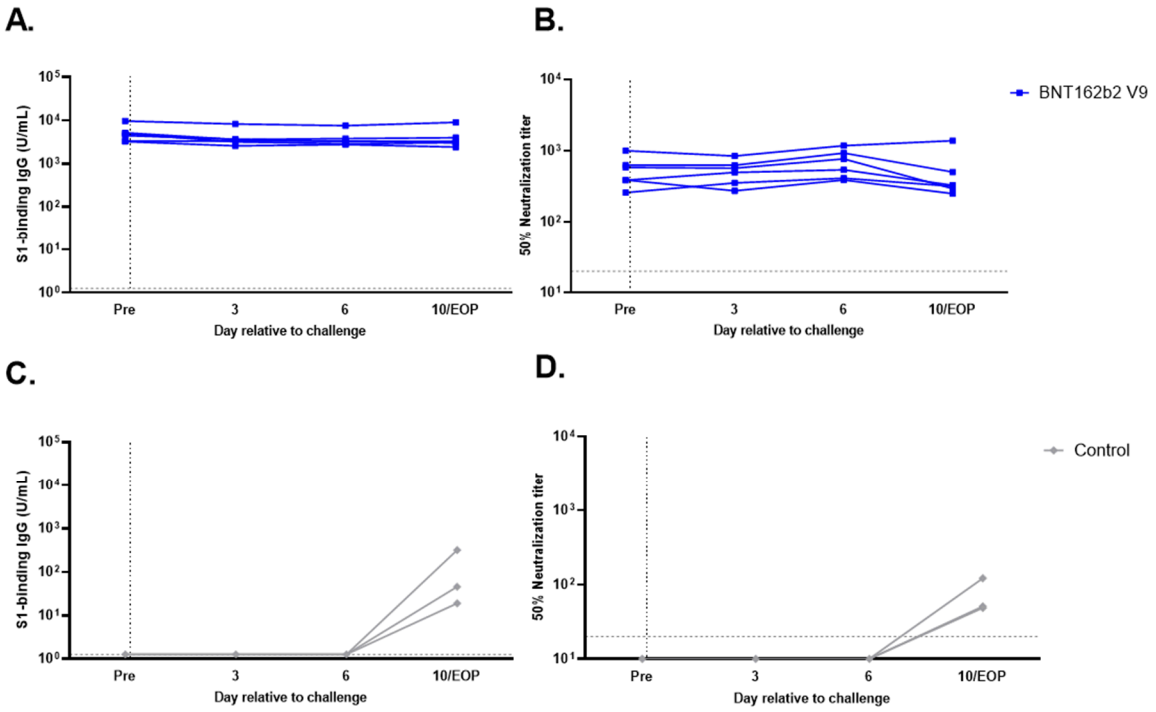
Figure 5. Viral RNA in BAL Fluid, Nasal Swabs, and Oropharyngeal Swabs of Rhesus Macaques after Infectious SARS-CoV-2 Challenge



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).

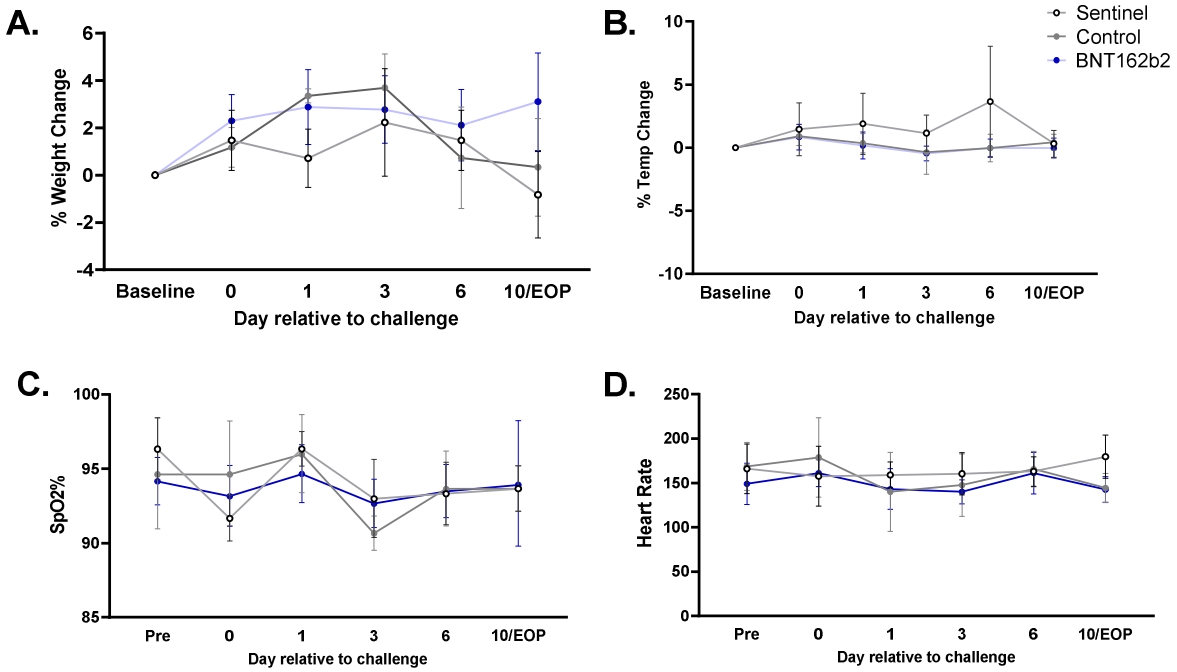
Figure 6. S1-binding IgG and 50% Serum Neutralization Responses in Rhesus Macaques after Infectious SARS-CoV-2 Challenge



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.

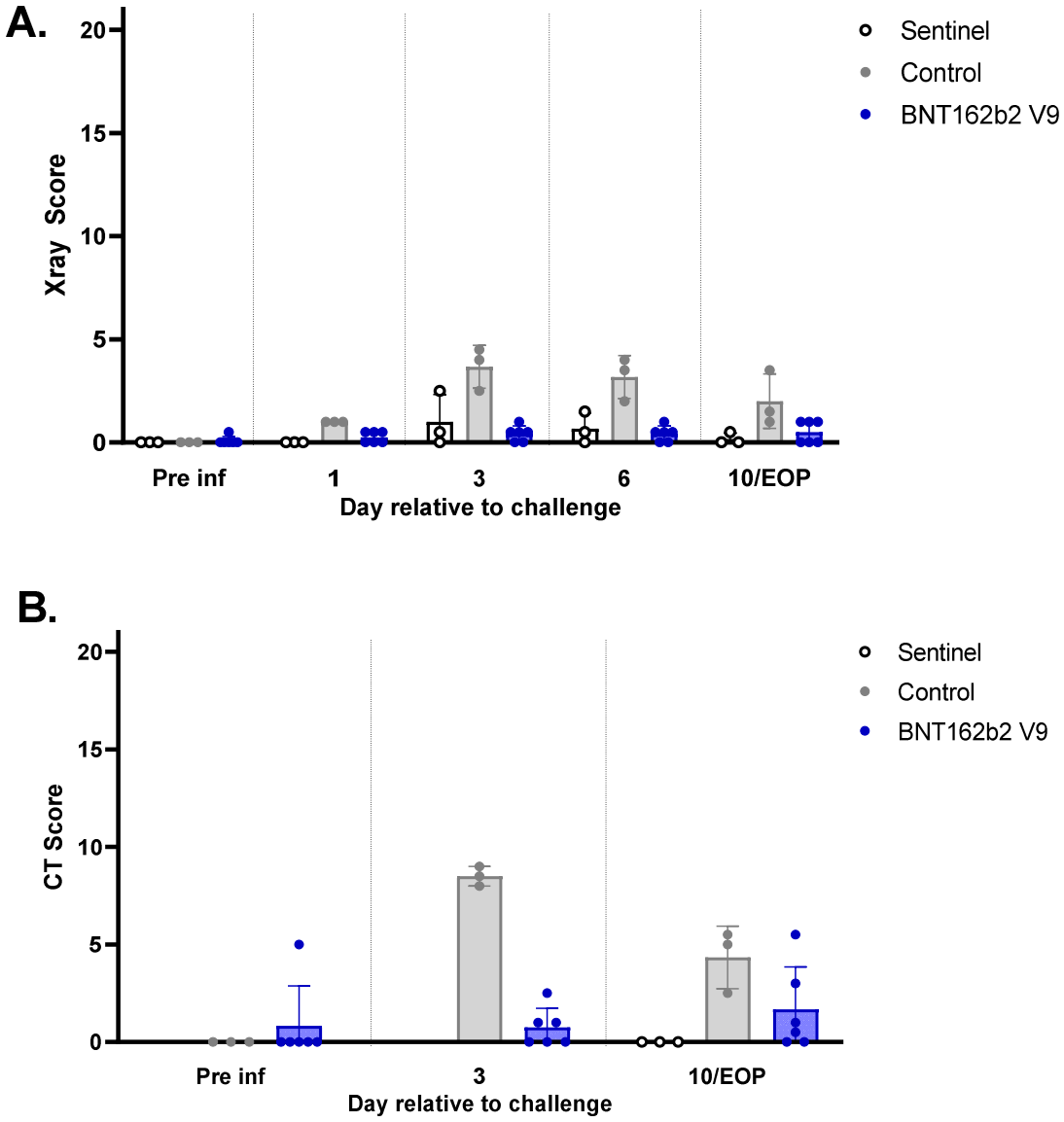
Despite the presence of viral RNA in BAL fluid from challenged control animals, none of the challenged animals, immunized or control, showed clinical signs of illness (Figure 7). Lung radiograph (Figure 8A) and computerized tomography (CT) (Figure 8B) scores were determined by two board-certified veterinary 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

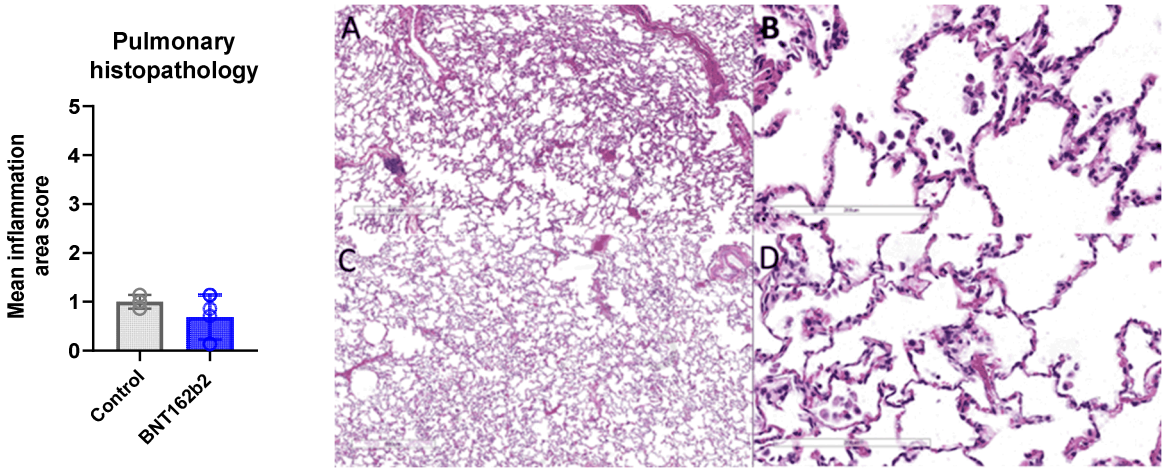


Fifty-five days after the second immunization, BNT162b2 or Control (saline)-immunized animals were challenged with 1.05×10^6 pfu of SARS-CoV-2 split equally between the IN and IT routes. Three age-matched unimmunized rhesus macaques were challenged with cell culture medium only (Sentinel). Chest X-rays 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

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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
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In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20008
Experimental Start Date	2020-06-04
Experimental Completion Date	2020-06-08
Number of Pages in Report	29

TABLE OF CONTENTS

SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0315 in liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 2-hour incubation with liver microsomes from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6) _____ 2020/08/03

Study Director Date

Sponsor Approval:

(b) (6) _____ August 3, 2020

Study Monitor Date

1. OBJECTIVE

To evaluate the in vitro metabolic stability of ALC-0315 in liver microsomes from different species.

2. MATERIALS

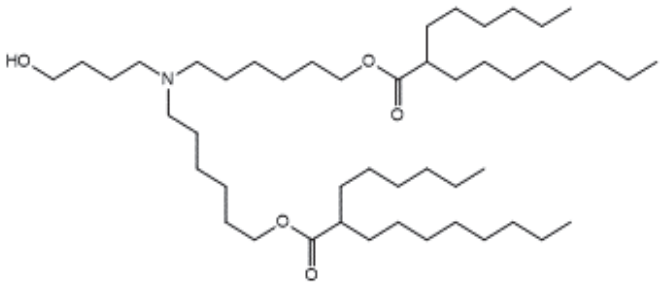
2.1 Test Article

Name: ALC-0315

Molecular Formula: $C_{48}H_{95}NO_5$

MW (g/mol): 766.27

Exact Mass: 765.72



2.2 Positive Control

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Ketanserin	TCI	74050-98-9	K0051	NPGAF-CO	395.43

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Verapamil hydrochloride	TCI	152-11-4	V0118	RFMWJ-RL	491.06

2.4 Liver Microsomes and Cofactor

The following pooled liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were stored in a -70°C ultra low temperature freezer prior to use.

Species	Manufacturer	Cat. No.	Lot No.	Protein Concentration (mg/mL)
CD-1/ICR mouse (male)	XenoTech	M1000	1910002	20
Sprague Dawley rat (male)	XenoTech	R1000	1910100	20
Wistar Han rat	BioIVT	BCF	201801BCF	20
Cynomolgus monkey (male)	RILD Shanghai	LM-SXH-02M	NXNN	20
Human (mixed gender)	XenoTech	H0610	1810003	20

2.5 Coenzyme

NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt was stored at 2-8°C in a refrigerator prior to use.

Compound Name	Manufacturer	Cat. No.	Molecular Weight	Purity
NADPH	Roche Diagnostic	10621706001	833.35	97%

3. EXPERIMENTAL PROCEDURES

3.1 Stock solution: 2.54 mg of ALC-0315 was weighed and dissolved in 331.48 µL of DMSO to obtain a 10 mM stock solution. 3.237 mg of ketanserin was weighed and dissolved in 818.60 µL of DMSO to obtain a 10 mM stock solution.

3.2 0.5 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution (mM)	Volume of stock solution (µL)	Volume of MeOH (µL)	Final Concentration (mM)
10	10	190	0.5

3.3 1.5× liver microsomes suspension containing test article or positive control:

1.5× Liver Microsomes Suspension Containing Test Article or Positive Control					
Liver Microsomes		0.5 mM spiking solution (μL)	100 mM potassium phosphate buffer (pH 7.4) (μL)	Final Concentration	
Conc. of stock suspension (mg/mL)	Volume of stock suspension (μL)			Liver microsomal protein (mg/mL)	Compound (μM)
20	18.75	1.5	479.75	0.75	1.5

3.4 22.98 mg of NADPH was weighed and dissolved in 4.596 mL of 100 mM potassium phosphate buffer to obtain a 6 mM NADPH working solution. This working solution was then pre-warmed at 37ºC.

3.5 30 μL of 1.5× liver microsomes suspension containing test article or positive control was added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

3.6 96-well incubation plates were pre-warmed at 37 ºC for 5 min.

3.7 For 0-min samples: 450 μL of ethanol containing internal standard (IS solution) was added before 15 μL of pre-warmed NADPH working solution (6 mM) was added.

3.8 For other samples (15, 30, 60, 90, and 120 min): 15 μL of pre-warmed NADPH working solution (6 mM) was added to initiate the reaction.

Volume (μL)			Final Concentration in Incubation Mixture		
1.5× Liver Microsomes Suspension Containing Test Article or Positive Control	3×NADPH working solution	Total	Liver microsomal protein (mg/mL)	Test Article or Positive Control (μM)	NADPH (mM)
30	15	45	0.5	1	2

The samples were incubated at 37 ºC and 450 μL of IS solution was added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

3.9 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuged at 6,000 rpm for 15 min.

3.10 200 μL of supernatant was transferred from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1 Instruments

Waters Acquity UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column：ACQUITY UPLC BEH HILIC 1.7 μm (2.1*100 mm)

Gradient for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

Solvent A: 10 mM ammonium formate, 0.1% formic acid in water

Solvent B: 10 mM ammonium formate, 0.1% formic acid in acetonitrile

Flow rate：500 μL/min

Column temperature：40 °C

Autosampler temperature: 4°C

MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time
ALC-0315	766.90	510.60	100	66	~1.13
Verapamil (IS)	455.30	165.20	49	28	~1.25

4.3 Detection of ALC-0315

Representative chromatograms of ALC-0315 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0315 or positive control, ketanserin) was calculated by dividing the peak area ratio (test article peak area/internal standard peak area) by the time zero

peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life was calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope

Half-life ($t_{1/2}$) = 0.693/k

The *in vitro* intrinsic clearance, CL'_{int}, was calculated from the $t_{1/2}$ as follows:

CL'_{int} = (0.693/$t_{1/2}$) × (1/ (microsomal protein concentration (0.5 mg/mL))) × Scaling Factor

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Liver Microsomes

Species	Microsomal Protein (mg) per Gram of Liver	Liver Weight (g) per kg Body Weight	Scaling Factor (mg/kg)[a]	Hepatic Blood Flow (mL/min/kg)
Mouse	45	87.5	3937.5	90
Rat	44.8	40	1792	55.2
Monkey	45	32.5	1462.5	44
Human	48.8	25.7	1254.2	20.7

[a]Microsomal protein (mg/g liver) × liver weight (g)/kg body weight

6. RESULTS

A summary of the % remaining parent compound, CL'_{int} and half-life of ALC-0315 obtained from a 2-hour incubation of ALC-0315 with liver microsomes from CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human is presented in Table 2. The stability of ALC-0315 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The liver microsomes used in this study were tested for activity using a metabolism control substrate under incubation conditions identical to those used for ALC-0315. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compound (ketanserin) during the 2-hour incubation period, hence the test systems were

considered to have yielded valid results. A summary of the % remaining parent compound, CL'_{int} and half-life of ketanserin is provided in Table 2. The stability of ketanserin over time in each matrix is shown in Figure 2. Raw data is presented in Appendix 3.

7. CONCLUSION

This study evaluated the *in vitro* metabolic stability of ALC-0315 in liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 2-hour incubation with liver microsomes from all these species.

Table 2. Summary of Liver Microsomal Stability of ALC-0315 and Ketanserin

Test Article	Species		Percent Remaining (%)						$t_{1/2}$ (minute)	CL'int (mL/min/kg)
			0 min	15 min	30 min	60 min	90 min	120 min		
ALC-0315	CD-1/ICR mouse	Mean	100	98.77	97.78	100.49	97.78	96.54	**>120**	<45.5
		RSD of Area Ratio	0.01	0.01	0.01	0	0.04	0.04		
	Sprague Dawley rat	Mean	100	94.39	96.26	99.73	98.66	95.99	**>120**	<20.7
		RSD of Area Ratio	0.05	0.05	0.05	0.05	0.03	0.04		
	Wistar Han rat	Mean	100	96.34	97.32	98.54	94.15	93.66	**>120**	<20.7
		RSD of Area Ratio	0.03	0.03	0.06	0.01	0.01	0.04		
	Cynomolgus monkey	Mean	100	97.96	96.18	100	97.96	97.71	**>120**	<16.9
		RSD of Area Ratio	0.05	0.03	0.01	0.02	0.03	0.03		
	Human	Mean	100	100.24	99.76	101.45	100.48	98.31	**>120**	<14.5
		RSD of Area Ratio	0.03	0.02	0.02	0.02	0.06	0.05		
Ketanserin	CD-1/ICR mouse	Mean	100	61.73	37.16	17.24*	10.16*	6.43*	**21.0**	260
		RSD of Area Ratio	0.04	0.01	0.02	0.05	0.01	0.05		
	Sprague Dawley rat	Mean	100	74.03	51.43	26.11	16.08*	10.01*	**30.7**	80.9
		RSD of Area Ratio	0.04	0.02	0.03	0.05	0.03	0.03		
	Wistar Han rat	Mean	100	54.03	25.10	6.76	2.35	1.18*	**16.4**	151
		RSD of Area Ratio	0.02	0.02	0.01	0.07	0.04	0.06		
	Cynomolgus monkey	Mean	100	71.44	47.42	24.00	13.05*	8.35*	**28.9**	70.1
		RSD of Area Ratio	0.03	0.02	0.01	0.02	0.04	0.02		
	Human	Mean	100	77.74	57.56	38.26	26.22*	24.46*	**43.1**	40.3
		RSD of Area Ratio	0.09	0.01	0.01	0.04	0.12	0.05		

* Compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked in * were in the slow disappearance phase and were excluded from half-life calculation.

Figure 1. Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes

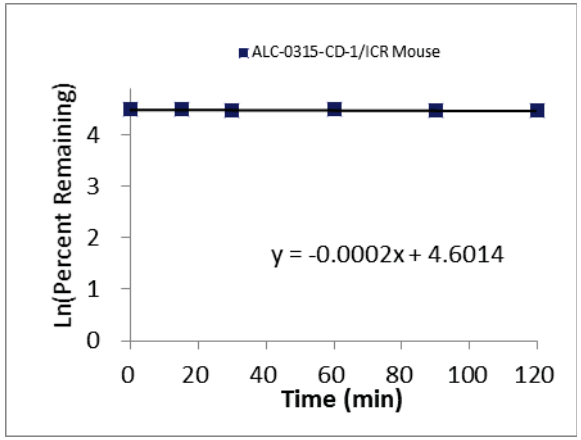
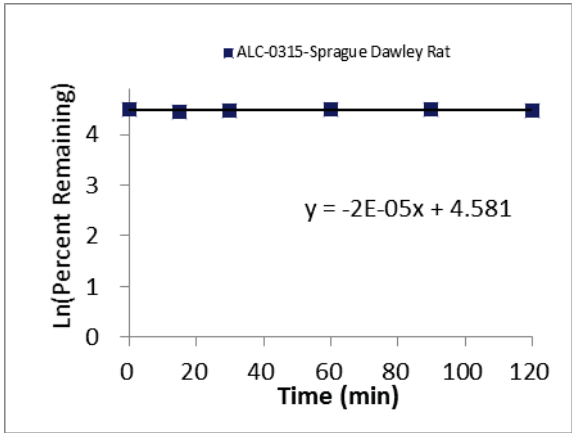
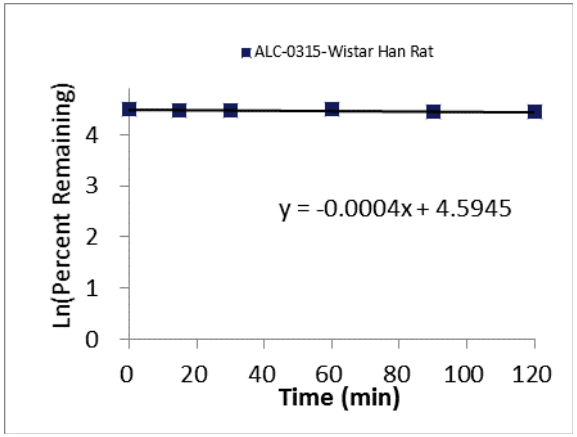
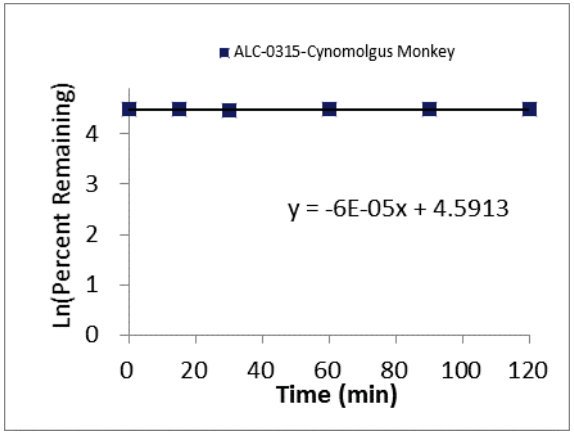
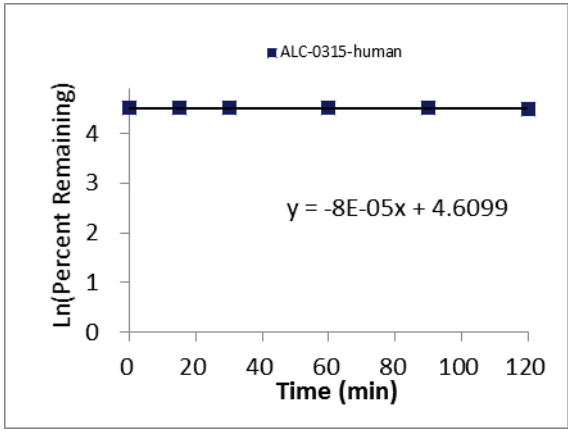
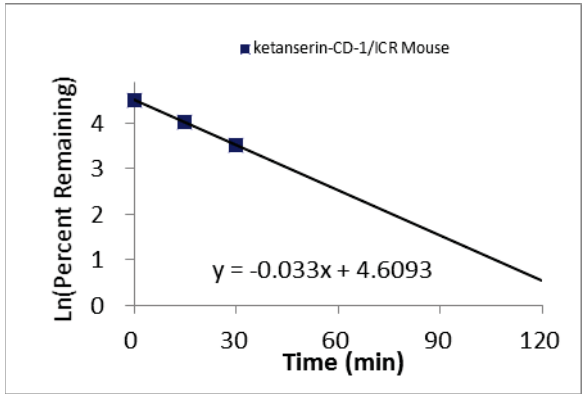
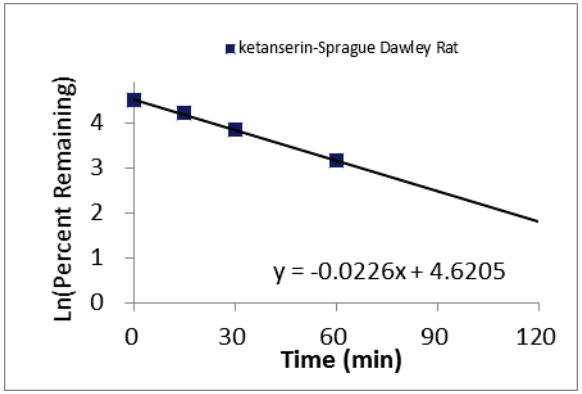
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 2. Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes

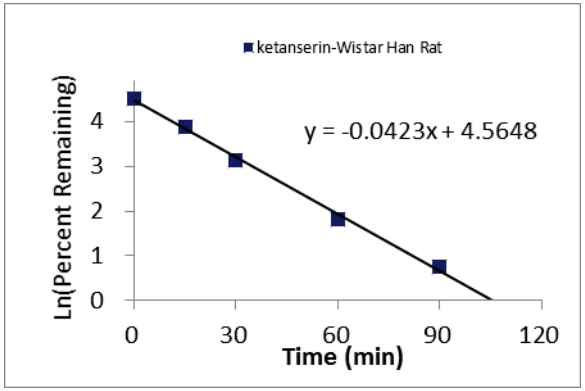
CD-1/ICR Mouse



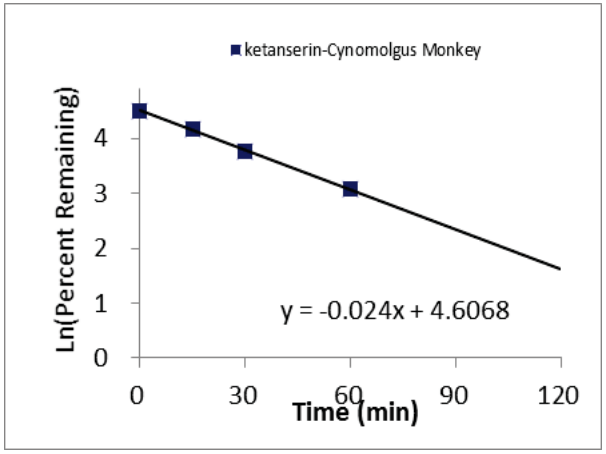
Sprague Dawley Rat



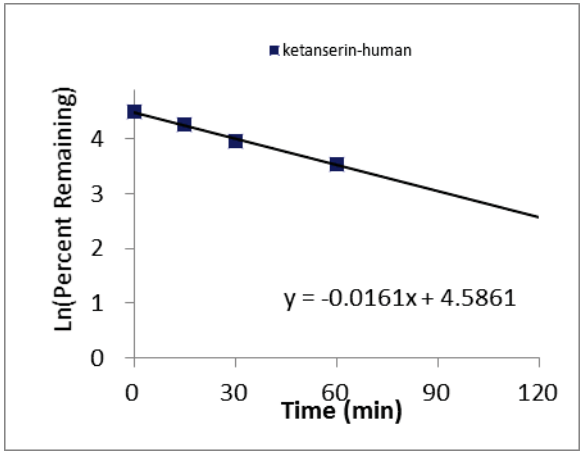
Wistar Han Rat



Cynomolgus Monkey



Human



8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes

Appendix 2 – Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

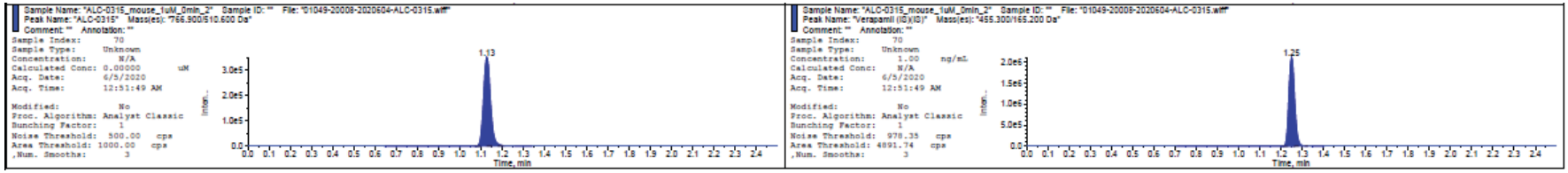
Appendix 3 – Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Appendix 4 –01049-20008-microsomal stability protocol

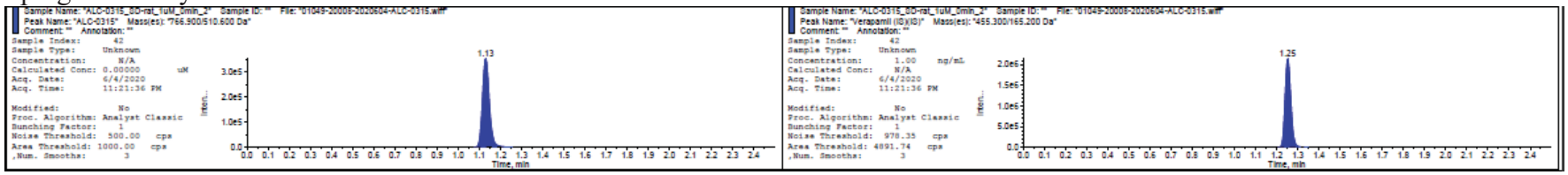
APPENDIX 1

Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes

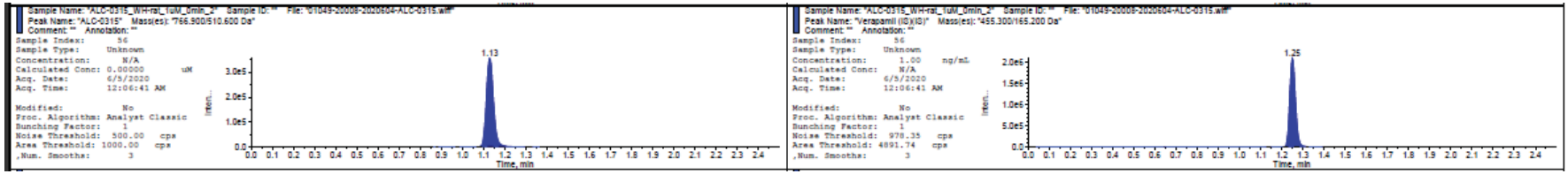
CD-1/ICR mouse



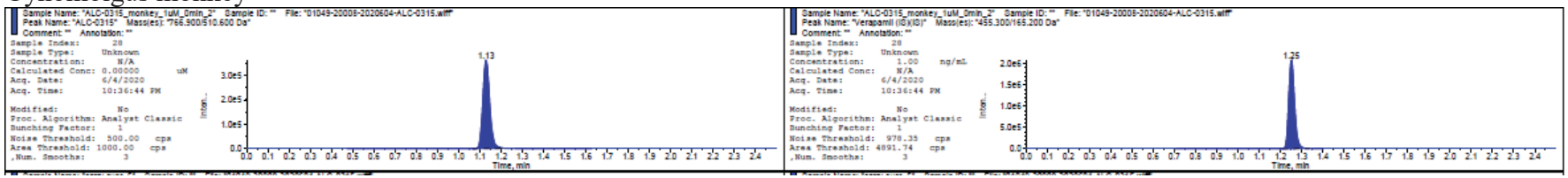
Sprague Dawley rat



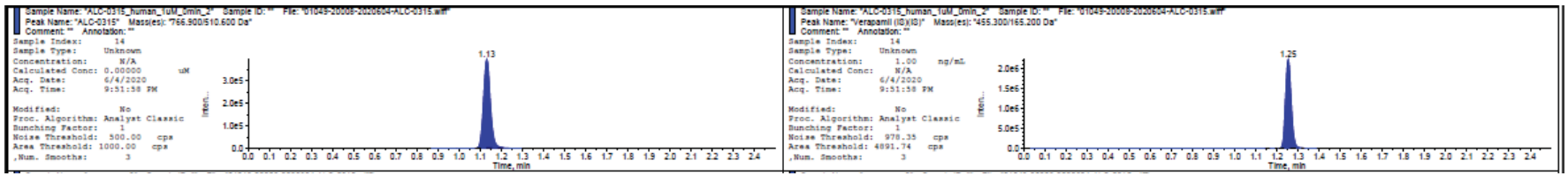
Wistar Han rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0315	CD-1/ICR mouse	0	8.00E+05	7.81E+05	3.91E+06	3.89E+06	0.20	0.20
		15	8.00E+05	7.73E+05	3.96E+06	3.91E+06	0.20	0.20
		30	7.80E+05	7.99E+05	3.99E+06	3.99E+06	0.20	0.20
		60	8.12E+05	8.41E+05	4.01E+06	4.11E+06	0.20	0.20
		90	7.79E+05	7.98E+05	4.04E+06	3.92E+06	0.19	0.20
		120	7.71E+05	7.76E+05	4.05E+06	3.86E+06	0.19	0.20
ALC-0315	Sprague Dawley rat	0	6.97E+05	7.73E+05	3.87E+06	3.98E+06	0.18	0.19
		15	6.87E+05	7.25E+05	4.04E+06	3.96E+06	0.17	0.18
		30	6.94E+05	7.47E+05	3.99E+06	4.01E+06	0.17	0.19
		60	7.16E+05	7.61E+05	3.99E+06	3.93E+06	0.18	0.19
		90	7.19E+05	7.55E+05	4.00E+06	4.00E+06	0.18	0.19
		120	6.82E+05	7.50E+05	3.93E+06	4.06E+06	0.17	0.19
ALC-0315	Wistar Han rat	0	7.65E+05	8.07E+05	3.81E+06	3.87E+06	0.20	0.21
		15	7.76E+05	8.05E+05	4.02E+06	3.98E+06	0.19	0.20
		30	7.59E+05	8.35E+05	3.98E+06	4.02E+06	0.19	0.21
		60	7.95E+05	8.05E+05	3.99E+06	3.95E+06	0.20	0.20
		90	7.80E+05	7.57E+05	4.06E+06	3.90E+06	0.19	0.19
		120	7.22E+05	8.17E+05	3.89E+06	4.12E+06	0.19	0.20
ALC-0315	Cynomolgus monkey	0	7.65E+05	8.06E+05	4.02E+06	3.97E+06	0.19	0.20
		15	7.65E+05	8.11E+05	4.07E+06	4.12E+06	0.19	0.20
		30	7.53E+05	7.62E+05	4.02E+06	3.98E+06	0.19	0.19
		60	7.80E+05	8.28E+05	4.01E+06	4.16E+06	0.19	0.20
		90	7.55E+05	8.13E+05	4.03E+06	4.13E+06	0.19	0.20
		120	7.87E+05	8.03E+05	4.18E+06	4.11E+06	0.19	0.20
ALC-0315	Human	0	7.90E+05	8.60E+05	3.90E+06	4.10E+06	0.20	0.21
		15	8.20E+05	8.50E+05	4.00E+06	4.10E+06	0.21	0.21
		30	8.20E+05	8.50E+05	4.00E+06	4.10E+06	0.20	0.21
		60	8.30E+05	8.50E+05	3.90E+06	4.10E+06	0.21	0.21
		90	8.60E+05	7.80E+05	4.00E+06	3.90E+06	0.22	0.20
		120	8.60E+05	8.00E+05	4.10E+06	4.10E+06	0.21	0.20

APPENDIX 3

Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Ketanserin	CD-1/ICR mouse	0	1.93E+06	1.99E+06	8.68E+05	8.45E+05	2.22	2.36
		15	1.18E+06	1.17E+06	8.32E+05	8.31E+05	1.42	1.41
		30	7.30E+05	7.08E+05	8.43E+05	8.45E+05	0.87	0.84
		60	3.42E+05	3.24E+05	8.37E+05	8.49E+05	0.41	0.38
		90	1.94E+05	1.94E+05	8.29E+05	8.36E+05	0.23	0.23
		120	1.20E+05	1.28E+05	8.43E+05	8.39E+05	0.14	0.15
Ketanserin	Sprague Dawley rat	0	2.00E+06	1.93E+06	8.58E+05	8.74E+05	2.33	2.21
		15	1.42E+06	1.46E+06	8.57E+05	8.57E+05	1.66	1.70
		30	9.99E+05	1.00E+06	8.34E+05	8.78E+05	1.20	1.14
		60	5.01E+05	5.15E+05	8.76E+05	8.37E+05	0.57	0.61
		90	3.16E+05	3.07E+05	8.43E+05	8.62E+05	0.37	0.36
		120	1.91E+05	1.89E+05	8.55E+05	8.14E+05	0.22	0.23
Ketanserin	Wistar Han rat	0	2.02E+06	2.08E+06	8.55E+05	8.52E+05	2.36	2.44
		15	1.08E+06	1.09E+06	8.41E+05	8.28E+05	1.28	1.31
		30	5.31E+05	5.23E+05	8.76E+05	8.71E+05	0.61	0.60
		60	1.29E+05	1.41E+05	8.41E+05	8.24E+05	0.15	0.17
		90	4.80E+04	4.97E+04	8.74E+05	8.55E+05	0.05	0.06
		120	2.31E+04	2.42E+04	8.56E+05	8.22E+05	0.03	0.03
Ketanserin	Cynomolgus monkey	0	2.07E+06	2.07E+06	8.64E+05	8.34E+05	2.40	2.49
		15	1.43E+06	1.46E+06	8.30E+05	8.23E+05	1.72	1.77
		30	9.68E+05	9.82E+05	8.42E+05	8.42E+05	1.15	1.17
		60	4.84E+05	4.88E+05	8.40E+05	8.18E+05	0.58	0.60
		90	2.68E+05	2.75E+05	8.65E+05	8.40E+05	0.31	0.33
		120	1.69E+05	1.65E+05	8.19E+05	8.19E+05	0.21	0.20
Ketanserin	Human	0	2.11E+06	1.97E+06	8.12E+05	8.57E+05	2.60	2.30
		15	1.57E+06	1.56E+06	8.30E+05	8.13E+05	1.89	1.92
		30	1.09E+06	1.19E+06	7.77E+05	8.37E+05	1.40	1.42
		60	7.23E+05	6.78E+05	7.52E+05	7.42E+05	0.96	0.91
		90	6.14E+05	5.18E+05	8.82E+05	8.80E+05	0.70	0.59
		120	4.40E+05	4.88E+05	7.60E+05	7.88E+05	0.58	0.62

APPENDIX 4

01049-20008-microsomal stability protocol

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road
Pudong, Shanghai 201299
China

Study Number
01049-20008

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20008

1.2. Study Title

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

1.3. Sponsor Representative

(b) (6)
Acuitas Therapeutics Inc.
6190 Agronomy Road, Suite 402
Vancouver BC V6T 1Z3
Canada
(b) (6)

1.4. Objective

To evaluate the *in vitro* metabolic stability of ALC-0315 in liver microsomes from different species and to determine intrinsic clearance in each species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

(b) (6)

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

2. MATERIALS

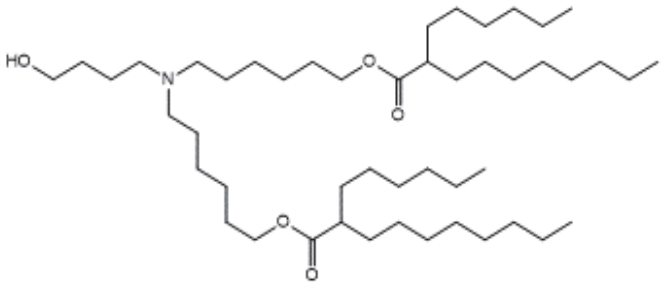
2.1. Test Article

Name: ALC-0315

Molecular Formula: $C_{48}H_{95}NO_5$

MW (g/mol): 766.27

Exact Mass: 765.72



2.2. Positive Control and Internal Standard

Ketanserin and verapamil will be used as positive control and internal standard, respectively. The sources will be documented in experimental records and presented in the report.

2.3. Liver Microsomes and Cofactor

Liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in a -70°C ultra low temperature freezer. NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt were purchased from a qualified supplier and stored at 2-8°C in a refrigerator. The source and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solution: Appropriate amount of test article or positive control is weighed and dissolved in DMSO to obtain a 10 mM stock solution.

(2) Preparation of 0.5 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution (mM)	Volume of stock solution (μL)	Volume of MeOH (μL)	Final Concentration (mM)
10	10	190	0.5

(3) Preparation of 1.5× liver microsomes suspension containing test article or positive control:

1.5× Liver Microsomes Suspension Containing Test Article or Positive Control					
Liver Microsomes		0.5 mM spiking solution (μL)	100 mM potassium phosphate buffer (pH 7.4) (μL)	Final Concentration	
Conc. of stock suspension (mg/mL)	Volume of stock suspension (μL)			Liver microsomal protein (mg/mL)	Compound (μM)
20	18.75	1.5	479.75	0.75	1.5

(4) 3×NADPH working solution (6 mM; 5 mg/mL) will be prepared by dissolving NADPH in 100 mM pH 7.4 potassium phosphate buffer. The working solution is then pre-warmed at 37°C.

(5) 30 μL of 1.5× liver microsomes suspension containing test article or positive control is added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

(6) 96-well incubation plates are pre-warmed at 37 °C for 5 min.

(7) For 0-min samples: 450 μL ethanol containing internal standard (IS solution) is added before 15 μL pre-warmed NADPH working solution (6mM) is added.

(8) For other samples (15, 30, 60, 90, and 120 min): 15 μL pre-warmed NADPH working solution (6 mM) is added to initiate reaction.

Volume (μL)			Final Concentration in incubation mixture		
1.5× Liver Microsomes Suspension Containing Test Article or Positive Control	3×NADPH working solution	Total	Liver microsomal protein (mg/mL)	Test Article or Positive Control (μM)	NADPH (mM)
30	15	45	0.5	1	2

The samples are incubated at 37 ºC and 450 µL IS solution is added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

(9) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(10) The plates are sealed and stored at -20 ºC in a freezer until bioanalysis.

(11) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 µL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

Waters Acquity UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column：ACQUITY UPLC BEH HILIC 1.7µm (2.1*100mm)

Gradient for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

A: 10mM ammonium formate, 0.1% Formic acid in water

B: 10mM ammonium formate, 0.1% Formic acid in acetonitrile

Flow rate：500 µL/min

Column temperature：40 ºC

Autosampler temperature: 4ºC

Compound	Q1(m/z)	Q3(m/z)	Retention Time (min)
ALC-0315	766.90	510.60	~1.07
Verapamil (IS)	455.30	165.20	~1.19

5. DATA ANALYSIS

The % remaining will be calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining will be plotted against time, and the slope of the regression line will be determined. Then elimination constant and half-life will be calculated as below.

$$\text{Elimination rate constant (k)} = - \text{ slope}$$

$$\text{Half-life } (t_{1/2}) = 0.693/k$$

The *in vitro* intrinsic clearance, CL'_{int}, will be calculated from the $t_{1/2}$ as follows:

$$CL'_{int} = (0.693/T_{1/2}) \times (1/(\text{microsomal protein concentration (0.5 mg/mL)})) \times \text{Scaling Factor}$$

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Liver Microsomes

Species	Microsomal Protein (mg) per Gram of Liver	Liver Weight (g) per kg Body Weight	Scaling Factor (mg/kg)[a]	Hepatic Blood Flow (mL/min/kg)
Mouse	45	87.5	3937.5	90
Rat	44.8	40	1792	55.2
Monkey	45	32.5	1462.5	44
Human	48.8	25.7	1254.2	20.7

[a]Microsomal protein (mg/g liver) × liver weight (g)/kg body weight

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

Sponsor Approval

(b) (6)

June 3, 2020
Date

Sponsor Representative

Study Director Approval

(b) (6)

2020/06/03
Date

Study Director

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20009
Experimental Start Date	2020-06-19
Experimental Completion Date	2020-06-24
Number of Pages in Report	31

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SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0315 in liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 2-hour incubation with liver S9 from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6)

Study Director

2020/08/10
Date

Sponsor Approval:

(b) (6)

Study Monitor

August 10, 2020
Date

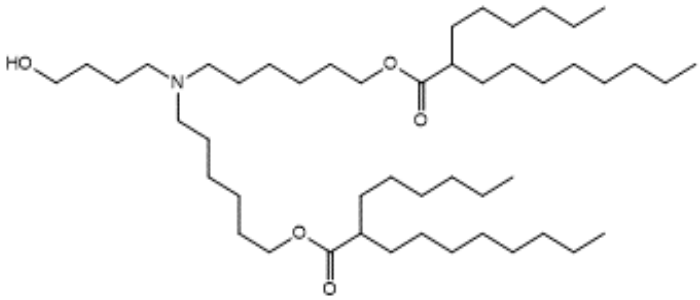
1. OBJECTIVE

To evaluate the *in vitro* metabolic stability of ALC-0315 in liver S9 fractions from different species.

2. MATERIALS

2.1 Test Article

Name: ALC-0315
Molecular Formula: $C_{48}H_{95}NO_5$
MW (g/mol): 766.27
Exact Mass: 765.72



2.2 Positive Controls

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Testosterone	Aladdin	58-22-0	T102169	K1505051	288.42
7-hydroxycoumarin	J&K Scientific	93-35-6	153384	L630I04	162.14

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Verapamil hydrochloride	TCI	152-11-4	V0118	RFMWJ-RL	491.06
Tolbutamide	Sigma-Aldrich	64-7-7	46968	BCBV8457	270.35

2.4 Liver S9 Fractions

The following pooled liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human were stored in a -70°C ultra low temperature freezer prior to use.

Species	Manufacturer	Cat. No.	Lot No.	Protein Concentration (mg/mL)
CD-1/ICR mouse (male)	XenoTech	M1000.S9	1310210	20
Sprague Dawley rat (male)	XenoTech	R1000.S9	1310212	20
Cynomolgus monkey (male)	XenoTech	P2000.S9	0910273	20
Human (mixed gender)	BioreclamationIVT	X008023	BKY	20

2.5 Coenzymes and Pore-forming Agent

NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt was stored at 2~8°C in a refrigerator prior to use. UDPGA (uridine-diphosphate-glucuronic acid trisodium salt) and alamethicin were stored in a -20°C freezer prior to use.

Compound Name	Manufacturer	Cat. No.	Molecular Weight	Purity
NADPH	Roche Diagnostic	10621706001	833.35	97%
UDPGA	Sigma	U6751	646.23	≥98
Alamethicin	Aladdin	A132913	1964.3078	99%

3. EXPERIMENTAL PROCEDURES

3.1 Stock solutions preparation:

2.54 mg of ALC-0315 was weighed and dissolved in 331.48 µL of DMSO to obtain a 10 mM stock solution. 2.547 mg of testosterone was weighed and dissolved in 551.93 µL of DMSO to obtain a 16 mM solution. A 10 mM testosterone stock solution was then prepared by adding 60 µL DMSO to 100 µL of the 16 mM testosterone solution. 3.97 mg of 7-hydroxycoumarin was weighed and dissolved in 1224.25 µL of DMSO to obtain a 20 mM solution. A 10 mM 7-hydroxycoumarin stock solution was then prepared by adding 100 µL DMSO to 100 µL of the 20 mM 7-hydroxycoumarin solution. 5 mg of alamethicin was weighed and dissolved in 495 µL of 100 mM potassium phosphate buffer to obtain a 10 mg/mL alamethicin solution.

3.2 0.5 mM spiking solutions preparation:

Spiking Solution of Test Article or Positive Control			
Conc. of Stock Solution (mM)	Volume of Stock Solution (µL)	Volume of MeOH (µL)	Final Concentration (mM)
10	10	190	0.5

3.3 Preparation of 1.5× liver S9 suspensions (with alamethicin) containing test article or positive control:

1.5× Liver S9 Suspension (with Alamethicin) Containing Test Article or Positive Control						
Livers S9		0.5 mM Spiking Solution (µL)	10 mg/ml Alamethicin Solution	100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$ (pH 7.4) (µL)	Final Concentration	
Conc. of Stock Solution (mg/mL)	Volume of Stock Solution (µL)				Liver S9 Protein (mg/mL)	Compound (µM)
20	37.5	1.5	1.9	459.1	1.5	1.5

3.4 1.5× liver S9 suspensions (with alamethicin) containing test article or positive control were kept on ice for 15 minutes.

3.5 28.51 mg of NADPH was weighed and dissolved in 2.851 mL of 100 mM potassium phosphate buffer to obtain a 12 mM NADPH working solution. 25 mg of UDPGA was weighed and dissolved in 6.448 mL of 100 mM potassium phosphate buffer to obtain a 6 mM UDPGA working solution. 2.8 mL of 12 mM NADPH working solution was added into 2.8 mL of 6 mM UDPGA working solution to obtain a 6 mM NADPH and 3 mM UDPGA mixed working solution. This mixed working solution was then pre-warmed at 37ºC.

3.6 30µL of liver S9 suspension (with alamethicin) containing 1.5 µM test article or positive control was added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

3.7 96-well incubation plates were pre-warmed at 37ºC for 5 min.

3.8 For 0 min samples: 450 µL of ethanol containing internal standard (IS solution) was added before 15 µL of pre-warmed mixed working solution (6 mM NADPH and 3 mM UDPGA) was added.

3.9 For other samples (15, 30, 60, 90, and 120 min): 15 µL of pre-warmed mixed working solution (6 mM NADPH and 3 mM UDPGA) was added to initiate the reaction.

Volume (µL)			Final Concentration in Incubation Mixture		
1.5× Liver S9 Suspension Containing Test Article or Positive Control	3×NADPH Working Solution	Total	Liver S9 Protein (mg/mL)	Test Article or Positive Control (µM)	NADPH (mM)
30	15	45	0.5	1	2

The samples were incubated at 37ºC and 450 µL of IS solution was added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

3.10 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuge at 6,000 rpm for 15 min.

3.11 Then 200 µL of the supernatant from each well was transferred into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1 Instruments

Waters Acquity UPLC system
Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column: ACQUITY UPLC BEH HILIC 1.7 µm (2.1*100 mm)
Gradient for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

Solvent A: 10mM ammonium formate, 0.1% formic acid in water
Solvent B: 10mM ammonium formate, 0.1% formic acid in acetonitrile
Flow rate： 500 µL/min
Column temperature： 40ºC
Autosampler temperature: 4ºC
MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time (min)
ALC-0315	766.90	510.60	100	66	~1.08
Verapamil (IS)	455.30	165.20	49	28	~1.21

4.3 Detection of ALC-0315

Representative chromatograms of ALC-0315 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0315 or positive control, testosterone and 7-hydroxycoumarin) was calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life were calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope
Half-life ($t_{1/2}$) = 0.693/k

6. RESULTS

A summary of the % remaining parent compound and half-life of ALC-0315 obtained from a 2-hour incubation with liver S9 from CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human is presented in Table 1. The stability of ALC-0315 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The liver S9 used in this study were tested for activity using metabolism control substrates under incubation conditions identical to those used for ALC-0315. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compounds (testosterone and 7-hydroxycoumarin) during the 2-hour incubation period, hence the test systems were considered to have yielded valid results. A summary of the % remaining parent compound and half-life of testosterone and 7-hydroxycoumarin is provided in Table 1. The stability of testosterone and 7-hydroxycoumarin over time in each matrix is shown in Figure 2 and Figure 3, respectively. Raw data for controls is presented in Appendix 3 (testosterone) and Appendix 4 (7-hydroxycoumarin).

7. CONCLUSIONS

This study evaluated the *in vitro* metabolic stability of ALC-0315 in liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 2-hour incubation with liver S9 from all these species.

Table 1. Summary of Liver S9 Stability of ALC-0315 , Testosterone and 7-Hydroxycoumarin

Compounds	Species		Percent Remaining (%)						$T_{1/2}$ (minute)
			0 min	15 min	30 min	60 min	90 min	120 min	
ALC-0315	CD-1/ICR Mouse	Mean	100.00	97.69	97.22	98.61	98.15	96.76	**>120**
		RSD of Area Ratio	0.03	0.03	0.01	0.02	0.00	0.02	
	Sprague Dawley Rat	Mean	100.00	98.85	99.62	99.62	98.85	98.46	**>120**
		RSD of Area Ratio	0.03	0.03	0.06	0.06	0.05	0.03	
	Cynomolgus Monkey	Mean	100.00	99.57	96.96	99.13	98.70	99.57	**>120**
		RSD of Area Ratio	0.04	0.02	0.01	0.01	0.01	0.01	
	Human	Mean	100.00	95.99	97.32	94.98	98.33	99.33	**>120**
		RSD of Area Ratio	0.06	0.03	0.04	0.00	0.04	0.05	
Testosterone	CD-1/ICR Mouse	Mean	100.00	59.38	35.71	6.89	BQL	BQL	**15.5**
		RSD of Area Ratio	0.05	0.11	0.01	0.16	N/A	N/A	
	Sprague Dawley Rat	Mean	100.00	BQL	BQL	BQL	BQL	BQL	**N/A**
		RSD of Area Ratio	0.05	N/A	N/A	N/A	N/A	N/A	
	Cynomolgus Monkey	Mean	100.00	81.12	68.58	45.76	27.19	16.74	**46.6**
		RSD of Area Ratio	0.11	0.11	0.02	0.06	0.02	0.08	
	Human	Mean	100.00	63.69	17.40	BQL	BQL	BQL	**11.9**
		RSD of Area Ratio	0.00	0.02	0.09	N/A	N/A	N/A	
7-Hydroxycoumarin	CD-1/ICR Mouse	Mean	100.00	40.06	16.68	3.57	1.89*	1.54*	**12.5**
		RSD of Area Ratio	0.00	0.11	0.02	0.17	0.18	0.20	
	Sprague Dawley Rat	Mean	100.00	71.60	48.00	26.17*	13.71*	11.92*	**28.3**
		RSD of Area Ratio	0.08	0.04	0.07	0.06	0.00	0.04	
	Cynomolgus Monkey	Mean	100.00	80.70	64.55	38.33	23.76	16.34	**44.8**
		RSD of Area Ratio	0.04	0.04	0.05	0.00	0.01	0.04	
	Human	Mean	100.00	69.01	43.17	19.16	8.29	3.68	**25.0**
		RSD of Area Ratio	0.02	0.00	0.04	0.03	0.03	0.12	

* The compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked with * were in the slow disappearance phase and were excluded from half-life calculation. BQL = Below quantification limit; N/A = not applicable

Figure 1. Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver S9

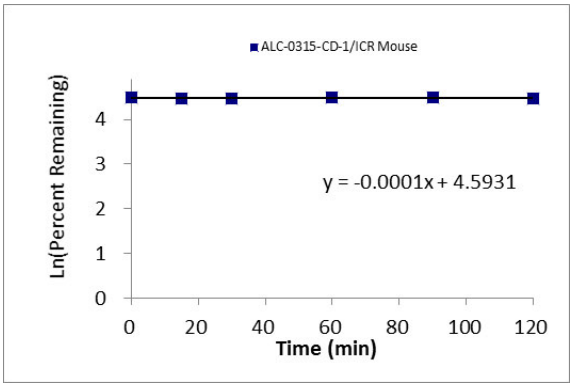
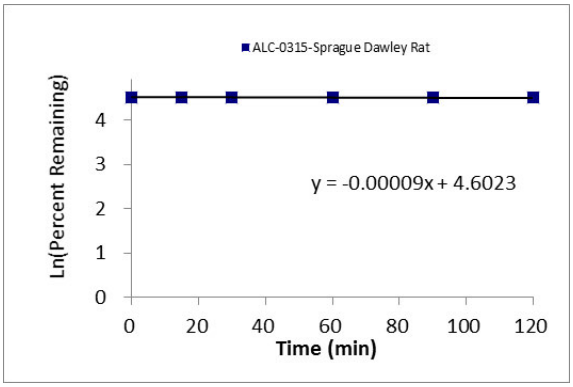
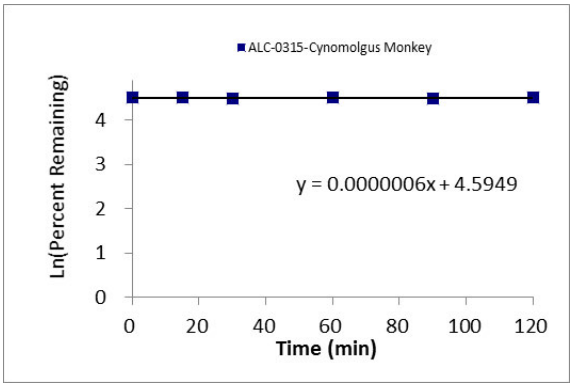
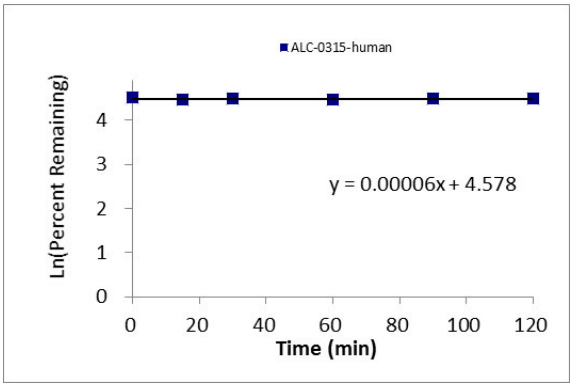
CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

Figure 2. Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9

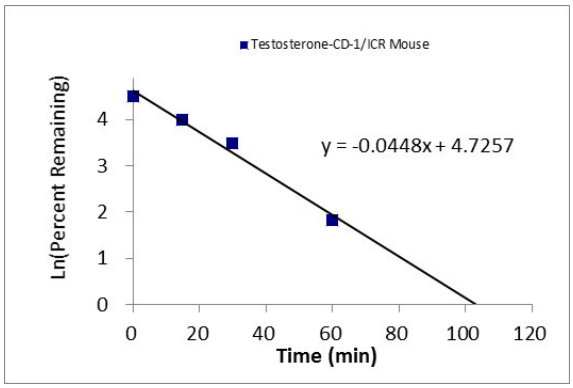
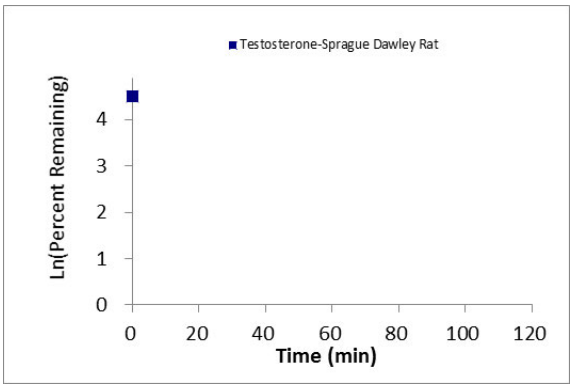
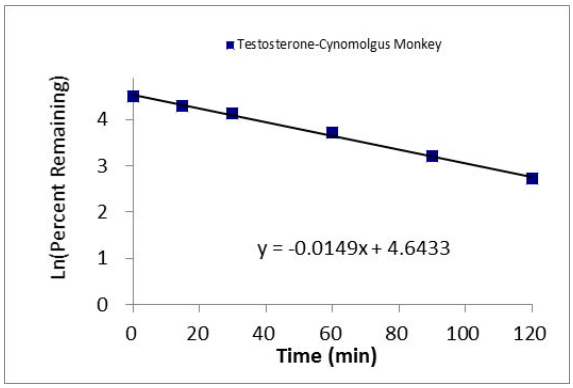
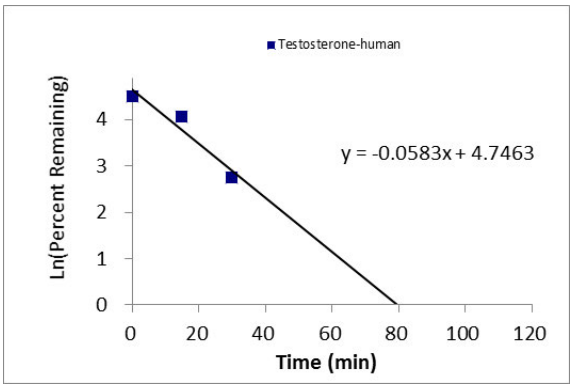
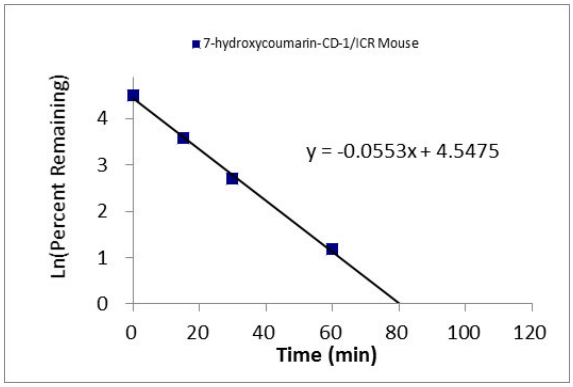
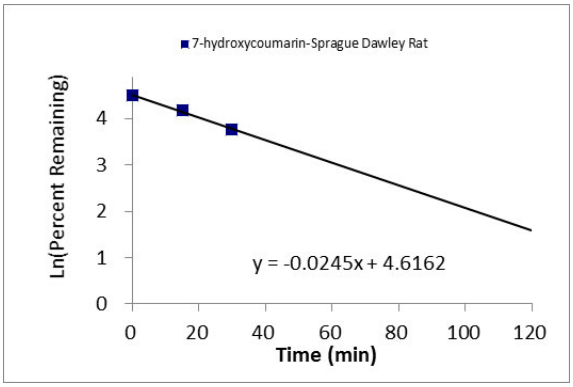
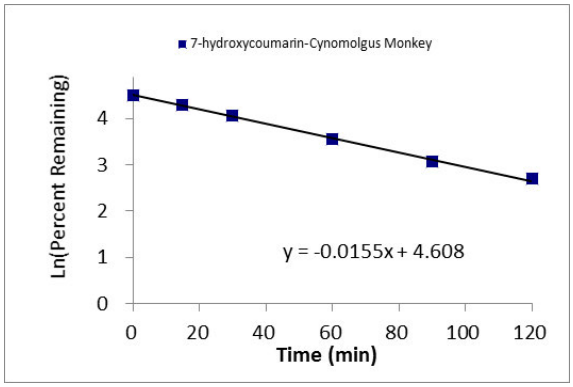
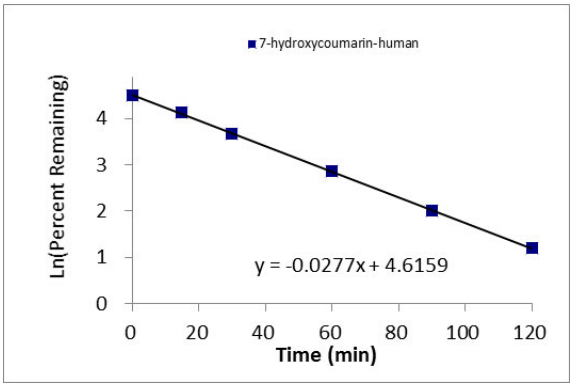
CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

Figure 3. Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9

CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Liver S9

Appendix 2 – Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Appendix 3 – Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

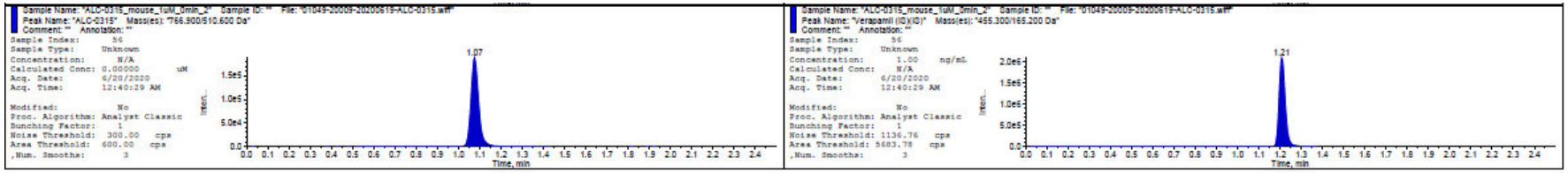
Appendix 4 – Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Appendix 5 – 01049-20009-S9 stability_protocol

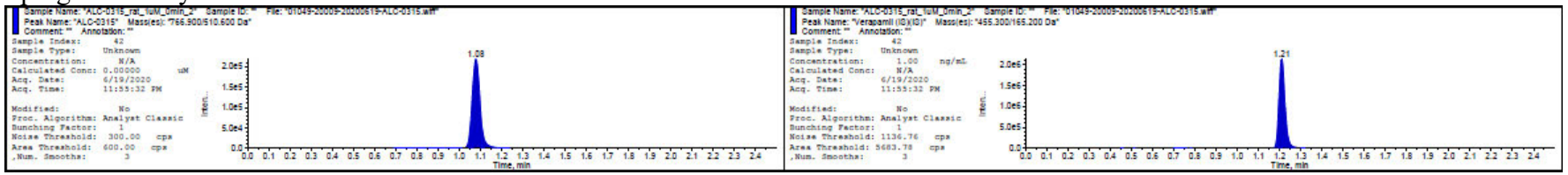
APPENDIX 1

Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Liver S9

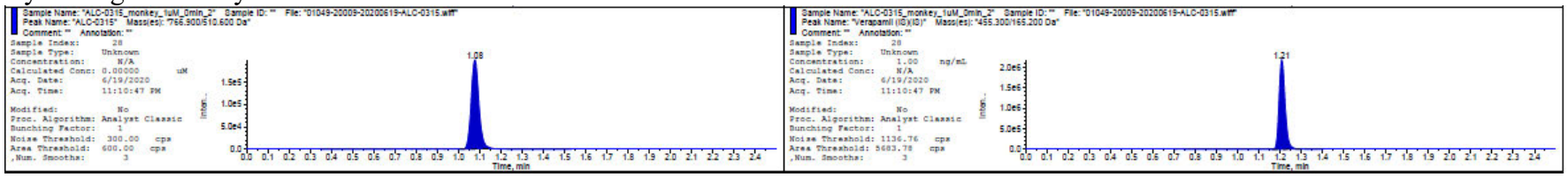
CD 1/ICR mouse



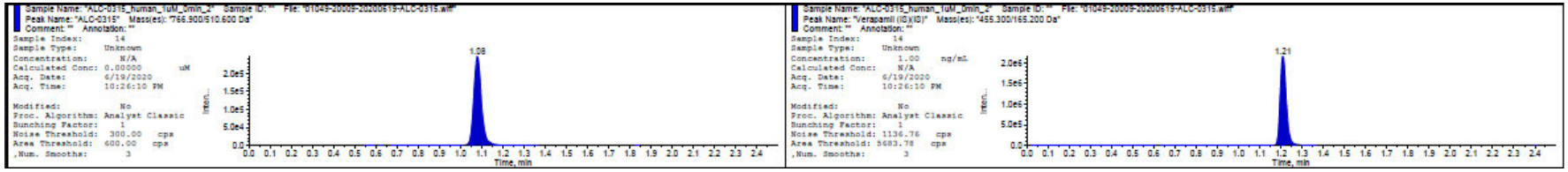
Sprague Dawley rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0315 in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0315	CD-1/ICR Mouse	0	4.33E+05	4.46E+05	4.08E+06	4.06E+06	0.106	0.110
		15	4.15E+05	4.49E+05	4.02E+06	4.15E+06	0.103	0.108
		30	4.21E+05	4.47E+05	4.06E+06	4.23E+06	0.104	0.106
		60	4.27E+05	4.48E+05	4.07E+06	4.15E+06	0.105	0.108
		90	4.47E+05	4.43E+05	4.23E+06	4.16E+06	0.106	0.106
		120	4.24E+05	4.44E+05	4.13E+06	4.17E+06	0.103	0.106
ALC-0315	Sprague Dawley Rat	0	5.23E+05	5.47E+05	4.12E+06	4.13E+06	0.127	0.133
		15	5.16E+05	5.37E+05	4.10E+06	4.11E+06	0.126	0.131
		30	5.10E+05	5.63E+05	4.12E+06	4.17E+06	0.124	0.135
		60	5.14E+05	5.59E+05	4.14E+06	4.15E+06	0.124	0.135
		90	5.22E+05	5.58E+05	4.20E+06	4.19E+06	0.124	0.133
		120	5.30E+05	5.50E+05	4.23E+06	4.22E+06	0.125	0.131
ALC-0315	Cynomolgus Monkey	0	4.57E+05	4.88E+05	4.07E+06	4.15E+06	0.112	0.118
		15	4.69E+05	4.90E+05	4.15E+06	4.21E+06	0.113	0.116
		30	4.60E+05	4.69E+05	4.13E+06	4.18E+06	0.111	0.112
		60	4.66E+05	4.81E+05	4.13E+06	4.19E+06	0.113	0.115
		90	4.73E+05	4.82E+05	4.18E+06	4.23E+06	0.113	0.114
		120	4.86E+05	4.83E+05	4.22E+06	4.23E+06	0.115	0.114
ALC-0315	Human	0	6.76E+05	6.00E+05	4.34E+06	4.20E+06	0.156	0.143
		15	6.28E+05	5.97E+05	4.27E+06	4.27E+06	0.147	0.140
		30	6.60E+05	6.02E+05	4.41E+06	4.26E+06	0.150	0.141
		60	6.17E+05	6.07E+05	4.34E+06	4.27E+06	0.142	0.142
		90	6.44E+05	6.03E+05	4.27E+06	4.21E+06	0.151	0.143
		120	6.44E+05	6.14E+05	4.17E+06	4.28E+06	0.154	0.143

APPENDIX 3

Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Testosterone	CD-1/ICR Mouse	0	1.74E+04	1.71E+04	5.35E+05	5.68E+05	0.032	0.030
		15	1.06E+04	1.16E+04	6.22E+05	5.77E+05	0.017	0.020
		30	6.44E+03	6.29E+03	5.72E+05	5.69E+05	0.011	0.011
		60	1.10E+03	1.37E+03	5.79E+05	5.69E+05	0.002	0.002
		90	LOD	LOD	5.84E+05	5.74E+05	LOD	LOD
		120	LOD	LOD	5.70E+05	5.75E+05	LOD	LOD
Testosterone	Sprague Dawley Rat	0	1.52E+04	1.62E+04	6.17E+05	6.14E+05	0.025	0.026
		15	LOD	LOD	6.05E+05	5.74E+05	LOD	LOD
		30	LOD	LOD	6.53E+05	5.96E+05	LOD	LOD
		60	LOD	LOD	5.75E+05	5.65E+05	LOD	LOD
		90	LOD	LOD	6.06E+05	5.80E+05	LOD	LOD
		120	LOD	LOD	5.98E+05	6.00E+05	LOD	LOD
Testosterone	Cynomolgus Monkey	0	1.68E+04	1.43E+04	5.88E+05	5.86E+05	0.029	0.024
		15	1.22E+04	1.32E+04	6.16E+05	5.72E+05	0.020	0.023
		30	1.08E+04	1.08E+04	6.04E+05	5.85E+05	0.018	0.018
		60	6.82E+03	7.12E+03	5.88E+05	5.64E+05	0.012	0.013
		90	4.29E+03	4.11E+03	5.87E+05	5.79E+05	0.007	0.007
		120	2.78E+03	2.38E+03	5.94E+05	5.69E+05	0.005	0.004
Testosterone	Human	0	1.66E+04	1.57E+04	5.95E+05	5.63E+05	0.028	0.028
		15	1.02E+04	1.05E+04	5.81E+05	5.81E+05	0.018	0.018
		30	3.05E+03	2.85E+03	5.88E+05	6.27E+05	0.005	0.005
		60	LOD	LOD	5.92E+05	5.75E+05	LOD	LOD
		90	LOD	LOD	6.07E+05	6.32E+05	LOD	LOD
		120	LOD	LOD	5.50E+05	5.76E+05	LOD	LOD

LOD = Limit of detection

APPENDIX 4

Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time (min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
7-Hydroxycoumarin	CD-1/ICR Mouse	0	2.69E+04	2.63E+04	5.42E+05	5.29E+05	0.050	0.050
		15	1.20E+04	1.01E+04	5.59E+05	5.52E+05	0.021	0.018
		30	4.62E+03	4.53E+03	5.49E+05	5.54E+05	0.008	0.008
		60	1.11E+03	8.20E+02	5.54E+05	5.27E+05	0.002	0.002
		90	5.99E+02	4.34E+02	5.62E+05	5.30E+05	0.001	0.001
		120	3.69E+02	4.87E+02	5.59E+05	5.58E+05	0.001	0.001
7-Hydroxycoumarin	Sprague Dawley Rat	0	2.60E+04	2.51E+04	5.20E+05	5.61E+05	0.050	0.045
		15	1.88E+04	1.82E+04	5.37E+05	5.52E+05	0.035	0.033
		30	1.29E+04	1.20E+04	5.39E+05	5.52E+05	0.024	0.022
		60	6.94E+03	6.43E+03	5.38E+05	5.40E+05	0.013	0.012
		90	3.57E+03	3.60E+03	5.49E+05	5.56E+05	0.007	0.006
		120	2.92E+03	3.11E+03	5.32E+05	5.34E+05	0.005	0.006
7-Hydroxycoumarin	Cynomolgus Monkey	0	2.46E+04	2.60E+04	5.32E+05	5.32E+05	0.046	0.049
		15	2.18E+04	2.06E+04	5.53E+05	5.50E+05	0.039	0.037
		30	1.65E+04	1.74E+04	5.57E+05	5.45E+05	0.030	0.032
		60	9.83E+03	9.77E+03	5.40E+05	5.35E+05	0.018	0.018
		90	6.29E+03	6.06E+03	5.52E+05	5.40E+05	0.011	0.011
		120	4.19E+03	4.03E+03	5.23E+05	5.34E+05	0.008	0.008
7-Hydroxycoumarin	Human	0	2.65E+04	2.65E+04	5.32E+05	5.16E+05	0.050	0.051
		15	1.88E+04	1.83E+04	5.37E+05	5.24E+05	0.035	0.035
		30	1.23E+04	1.15E+04	5.49E+05	5.42E+05	0.022	0.021
		60	5.42E+03	5.06E+03	5.48E+05	5.33E+05	0.010	0.009
		90	2.28E+03	2.37E+03	5.55E+05	5.52E+05	0.004	0.004
		120	9.57E+02	1.11E+03	5.60E+05	5.53E+05	0.002	0.002

APPENDIX 5

01049-20009-S9 stability_protocol

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong
Shanghai 201299, China

Study Number
01049-20009

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20009

1.2. Study Title

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

1.3. Sponsor Representative

(b) (6)

Acuitas Therapeutics Inc.

6190 Agronomy Road, Suite 402

Vancouver BC V6T 1Z3

Canada

(b) (6)

1.4. Objective

To evaluate the *in vitro* metabolic stability of ALC-0315 in liver S9 from different species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

[redacted]

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

2. MATERIALS

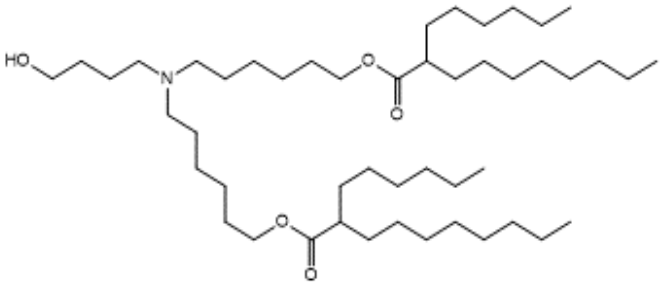
2.1. Test Article

Name: ALC-0315

Molecular Formula: $C_{48}H_{95}NO_5$

MW (g/mol): 766.27

Exact Mass: 765.72



2.2. Positive Control and Internal Standard

Testosterone and 7-hydroxycoumarin will be used as positive controls. Verapamil will be used as internal standard. The sources will be documented in experimental records and presented in the report.

2.3. Liver Microsomes and Cofactor

Liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey and human were purchased from qualified supplier and stored in a -70ºC ultra low temperature freezer.

NADPH (reduced β-Nicotinamide adenine dinucleotide 2′-phosphate tetrasodium salt) were purchased from qualified supplier and stored at 2-8ºC in a refrigerator.

UDPGA (uridine-diphosphate-glucuronic acid trisodium salt) and alamethicin were purchased from qualified suppliers and stored in a -20ºC freezer.

The source and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solutions: Appropriate amount of test article or positive control is weighed and dissolved in DMSO to obtain a 10 mM stock solution.

(2) Preparation of 0.5 mM spiking solutions:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution	Volume of stock solution (µL)	Volume of MeOH (µL)	Final Concentration
10 mM	10	190	0.5 mM

(3) Preparation of 1.5× liver S9 suspensions with alamethicin containing test article or positive control:

1.5× Liver S9 Suspension with Alamethicin containing Test article or Positive control						
Livers S9		0.5 mM spiking solution (µL)	10 mg/ml Alamethicin	100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$ (pH 7.4) (µL)	Final Concentration	
Conc. of stock solution (mg/mL)	Volume of stock solution (µL)				Liver S9 protein (mg/mL)	Compound (µM)
20	37.5	1.5	1.9	459.1	1.5	1.5

(4) 1.5× liver S9 suspensions with alamethicin containing test article or positive control are kept on ice for 15 minutes.

(5) 3× master mix of cofactors (6 mM NADPH and 3 mM UDPGA in 100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$, pH7.4) is prepared and then pre-warmed to 37ºC.

(6) 30 µL of liver S9 suspension with alamethicin containing 1.5 µM test article or positive control is added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

(7) 96-well incubation plates (from Step 6) are pre-warmed at 37 ºC for 5 min.

(8) For 0-min samples: 450 µL ethanol containing internal standard (IS solution) is added, followed by 15 µL pre-warmed 3× master mix of cofactors.

(9) For the 15, 30, 60, 90, and 120 min samples, 15 µL pre-warmed 3× master mix of cofactors is added to initiate reaction.

Volume of final incubation system (µL)			Final Concentration			
1.5× Liver S9 Suspension with Alamethicin containing Test article or Positive control	3× Master Mix of Cofactors	Total Volume	Liver S9 protein (mg/mL)	Compound (µM)	UDPGA (mM)	NADPH (mM)
30	15	45	1	1	1	2

The samples are incubated at 37 ºC and 450 µL IS solution (10ng/mL verapamil in ethanol) is added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

(10) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(11) The plates are sealed and stored at -20 ºC in a freezer until bioanalysis.

(12) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 µL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

Waters Acquity UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column：ACQUITY UPLC BEH HILIC 1.7µm (2.1*100mm)

Gradient Chromatography Parameters for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

Solvent A: 10mM ammonium formate, 0.1% Formic acid in water

Solvent B: 10mM ammonium formate, 0.1% Formic acid in acetonitrile

Flow rate：500 μL/min

Column temperature：40 ºC

Autosampler temperature: 4ºC

MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention
ALC-0315	766.90	510.60	100	66	~1.07
Verapamil	455.30	165.20	49	28	~1.19

5. DATA ANALYSIS

The % remaining will be calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining will be plotted against time, and the slope of the regression line will be determined. Then elimination constant and half-life will be calculated as below.

Elimination rate constant (k) = - slope

Half-life ($t_{1/2}$) = 0.693/k

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

Sponsor Approval

(b) (6)

June 17, 2020

Sponsor Representative

Date

Study Director Approval

(b) (6)

2020/06/17

Study Director

Date

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20010
Experimental Start Date	2020-07-20
Experimental Completion Date	2020-07-22
Number of Pages in Report	32

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SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0315 in hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 4-hour incubation with hepatocytes from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6)

Study Director

2020/08/10
Date

Sponsor Approval:

(b) (6)

Study Monitor

August 10, 2020
Date

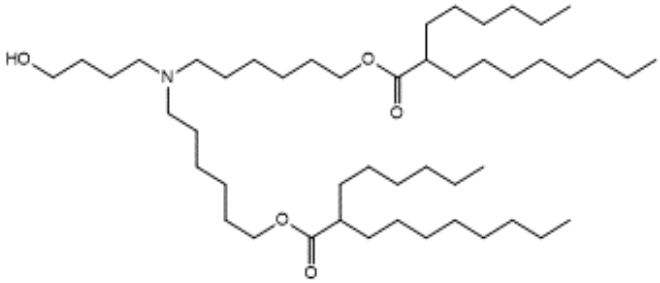
1. OBJECTIVE

To evaluate the *in vitro* metabolic stability of ALC-0315 in hepatocytes from different species.

2. MATERIALS

2.1 Test Article

Name: ALC-0315
Molecular Formula: $C_{48}H_{95}NO_5$
MW (g/mol): 766.27
Exact Mass: 765.72



2.2 Positive Control

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Testosterone	Aladdin	58-22-0	T102169	K1505051	288.42
7-hydroxycoumarin	J&K Scientific	93-35-6	153384	L630I04	162.14

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Verapamil hydrochloride	TCI	152-11-4	V0118	RFMWJ-RL	491.06
Tolbutamide	Sigma-Aldrich	64-7-7	46968	BCBV8457	270.35

2.4 Hepatocytes

The following cryopreserved hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in liquid nitrogen until use.

Species	Manufacturer	Cat. No.	Lot No.	Assured Minimum Yield (cells per vial)
CD-1/ICR mouse (male)	XenoTech	MPCH1000	1810242	2.0×10^6
Sprague Dawley rat (male)	XenoTech	RPCH1000	1810189	5.0×10^6
Wistar Han rat	BioIVT	M00065	YMV	5.0×10^6
Cynomolgus monkey (male)	RILD Shanghai	HP-SXH-02M	CJJC	5.0×10^6
Human (mixed gender)	XenoTech	HPCH10	1810156	5.0×10^6

3. EXPERIMENTAL PROCEDURES

3.1 Stock solution:

2.06 mg of ALC-0315 was weighed and dissolved in 268.83 µL of DMSO to obtain a 10 mM stock solution. 3.31 mg of testosterone was weighed and dissolved in 1147.60 µL of DMSO to obtain a 10 mM stock solution. 2.81 mg of 7-hydroxycoumarin was weighed and dissolved in 882.70 µL of DMSO to obtain a 10 mM stock solution.

3.2 4 mM spiking solution:

Spiking Solution of Test Article or Positive Control				
Compound	Conc. of Stock Solution (mM)	Volume of Stock Solution (µL)	Volume of DMSO (µL)	Final Concentration (mM)
ALC-0315	10	20	30	4
Testosterone & 7-Hydroxycoumarin	10	20	10	4

3.3 2 µM dosing solution (2×):

Dosing Solution (2×) of Test Article or Positive Control			
Conc. of Spiking Solution (mM)	Volume of Spiking Solution (µL)	Volume of William's E Medium (µL)	Final Concentration (µM)
4	2	3998	2

3.4 Preparation of hepatocyte suspension:

Cryopreserved hepatocytes were thawed in a 37°C water bath, transferred to hepatocyte thawing medium (William's E Medium with 30% percoll and 5% FBS), and then centrifuged at 100×g for 10 min at room temperature. The cell pellet was resuspended with William's E

Medium, cell viability was determined by trypan blue exclusion analysis, and the density of viable cells was calculated. The hepatocytes were diluted with incubation medium to an appropriate density (2×10^6 viable cells/mL) and then pre-warmed at 37 °C for 10 min.

3.5 40 µL of each hepatocyte suspension was added to 96-well plates in duplicate for each time point (0, 30, 60, 90, 120, 180, and 240 min).

3.6 For 0 min samples: 480 µL of internal standard solution (IS solution, 10 ng/mL verapamil in ethanol) was added, followed by 40 µL of pre-warmed 2× dosing solution. The final concentration of test article or positive control in the incubation mixture was 1 µM.

3.7 For the 30, 60, 90, 120, 180, and 240 min samples, 40 µL of pre-warmed 2× dosing solution was added to initiate the reaction. The final concentration of test article or positive control in the incubation mixture was 1 µM.

3.8 Samples were incubated at 37 ℃ . At 30, 60, 90, 120, 180, and 240 min time points, the reaction was stopped by adding 480 µL ethanol containing internal standard to all of the duplicate wells.

3.9 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuged at 6,000 rpm for 15 min.

3.10 The plates were sealed and stored at -20 °C until bioanalysis.

3.11 Plates were thawed at room temperature, centrifuged at 6,000 rpm for 15 min, and 200 µL of the supernatants were transferred from each well into a 96-well sample plate for LC-MS/MS.

4. BIOANALYSIS

4.1 Instruments

Waters Acuity UPLC system
Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column: ACQUITY UPLC BEH HILIC 1.7 µm (2.1*100 mm)
Gradient for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

Solvent A: 10mM ammonium formate, 0.1% formic acid in water
Solvent B: 10mM ammonium formate, 0.1% formic acid in acetonitrile
Flow rate：500 μL/min
Column temperature：40 ºC
Autosampler temperature: 4ºC
MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time
ALC-0315	766.90	510.60	100	66	~1.21
Verapamil (IS)	455.30	165.20	49	28	~1.32

4.3 Detection of ALC-0315

Representative chromatograms of ALC-0315 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0315 or positive control, testosterone and 7-hydroxycoumarin) was calculated by dividing the peak area ratio (test article peak area/internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life was calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope
Half-life (T1/2) (minutes) = 0.693/k
Intrinsic clearance, predicted from the *in vitro* hepatocyte stability study, was calculated as shown below:

CL'int (mL/min/kg) = k × V (1 mL incubation/10^6 cells) × Scaling Factor (10^6 cells/kg),
Scaling Factor (10^6 cells/kg) = Hepatocellularity (10^6 cells/g liver) × Normalized Liver Weight (g liver/kg body weight)

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Hepatocytes

Species	Hepatocellularity (10^6 cells/g liver)	Liver Weight (g/kg BW)	Scaling Factor (10^6 cells/kg)
Mouse	135	87.5	11812.5
Rat	117	40	4680
Monkey	120	32	3840
Human	99	25.7	2544.3

6. RESULTS

A summary of the % remaining parent compound, CL'_{int} and half-life of ALC-0315 obtained from a 4-hour incubation with hepatocytes from CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human is presented in Table 2. The stability of ALC-0315 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The hepatocytes used in this study were tested for activity using metabolism control substrates under incubation conditions identical to those used for ALC-0315. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compounds (testosterone and 7-hydroxycoumarin) during the 4-hour incubation period, hence the test systems were considered to have yielded valid results. A summary of the % remaining parent compound, CL'_{int} and half-life of testosterone and 7-hydroxycoumarin is provided in Table 2. The stability of testosterone and 7-hydroxycoumarin over time in each matrix is shown in Figure 2 and Figure 3, respectively. Raw data for controls is presented in Appendix 3 (testosterone) and Appendix 4 (7-hydroxycoumarin).

7. CONCLUSIONS

This study evaluated the *in vitro* metabolic stability of ALC-0315 in hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0315 was stable after an approximately 4-hour incubation with hepatocytes from all these species.

Table 2. Summary of Hepatocyte Stability of ALC-0315, Testosterone and 7-Hydroxycoumarin

Test Article	Species		Percent Remaining (%)							$T_{1/2}$ (minute)	CL'int (mL/min/kg)
			0 min	30 min	60 min	90 min	120 min	180 min	240 min		
ALC-0315	CD-1/ICR mouse	Mean	100.00	101.15	100.77	101.92	98.85	101.15	99.62	**>240**	**<34.1**
		RSD of Area Ratio	1.63	1.07	0.54	0.00	2.19	0.00	1.09		
	Sprague Dawley rat	Mean	100.00	97.75	98.50	99.25	97.38	98.88	101.12	**>240**	**<13.5**
		RSD of Area Ratio	1.59	3.79	3.76	1.60	2.18	4.29	2.10		
	Wistar Han rat	Mean	100.00	102.70	102.32	103.09	99.61	103.47	100.00	**>240**	**<13.5**
		RSD of Area Ratio	0.55	1.06	1.60	0.53	1.10	3.17	7.10		
	Cynomolgus monkey	Mean	100.00	96.36	97.82	100.00	96.36	95.64	93.82	**>240**	**<11.3**
		RSD of Area Ratio	1.54	1.60	2.63	3.60	3.74	1.61	4.39		
	Human	Mean	100.00	100.72	101.44	100.36	100.72	98.92	99.64	**>240**	**<7.35**
		RSD of Area Ratio	2.03	1.01	3.01	2.53	0.00	0.51	0.51		
Testosterone	CD-1/ICR mouse	Mean	100.00	16.60	BQL	BQL	BQL	BQL	BQL	**11.6**	**707**
		RSD of Area Ratio	5.81	11.78	N/A	N/A	N/A	N/A	N/A		
	Sprague Dawley rat	Mean	100.00	7.23	BQL	BQL	BQL	BQL	BQL	**7.92**	**410**
		RSD of Area Ratio	3.17	N/A	N/A	N/A	N/A	N/A	N/A		
	Wistar Han rat	Mean	100.00	BQL	BQL	BQL	BQL	BQL	BQL	**N/A**	**N/A**
		RSD of Area Ratio	8.03	N/A	N/A	N/A	N/A	N/A	N/A		
	Cynomolgus monkey	Mean	100.00	10.07	BQL	BQL	BQL	BQL	BQL	**9.06**	**298**
		RSD of Area Ratio	2.81	41.26	N/A	N/A	N/A	N/A	N/A		
	Human	Mean	100.00	15.92	BQL	BQL	BQL	BQL	BQL	**11.3**	**156**
		RSD of Area Ratio	4.34	7.16	N/A	N/A	N/A	N/A	N/A		

7-Hydroxycoumarin	CD-1/ICR mouse	Mean	100	35.05	3.2	BQL	BQL	BQL	BQL	**12.1**	**677**
		RSD of Area Ratio	1.22	15.06	8.46	N/A	N/A	N/A	N/A		
	Sprague Dawley rat	Mean	100	20.97	BQL	BQL	BQL	BQL	BQL	**13.3**	**244**
		RSD of Area Ratio	2.99	10.49	N/A	N/A	N/A	N/A	N/A		
	Wistar Han rat	Mean	100	19.11	BQL	BQL	BQL	BQL	BQL	**12.6**	**258**
		RSD of Area Ratio	1.97	16.89	N/A	N/A	N/A	N/A	N/A		
	Cynomolgus monkey	Mean	100	17.03	BQL	BQL	BQL	BQL	BQL	**11.7**	**230**
		RSD of Area Ratio	0.85	2.27	N/A	N/A	N/A	N/A	N/A		
	Human	Mean	100	40.7	18.53	3.36	BQL	BQL	BQL	**24.7**	**71.5**
		RSD of Area Ratio	1.52	1.67	8.47	0.73	N/A	N/A	N/A		

* Compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked in * were in the slow disappearance phase and were excluded from half-life calculation.
BQL = Below quantification limit; N/A = not applicable

Figure 1. Stability of ALC-0315 in Mouse, Rat, Monkey and Human Hepatocytes

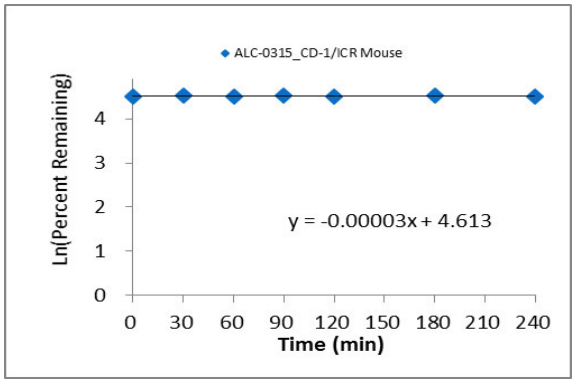
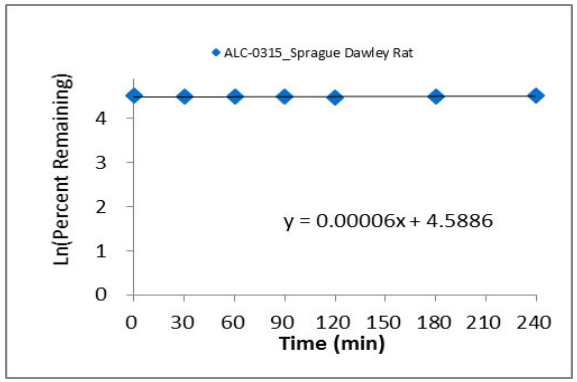
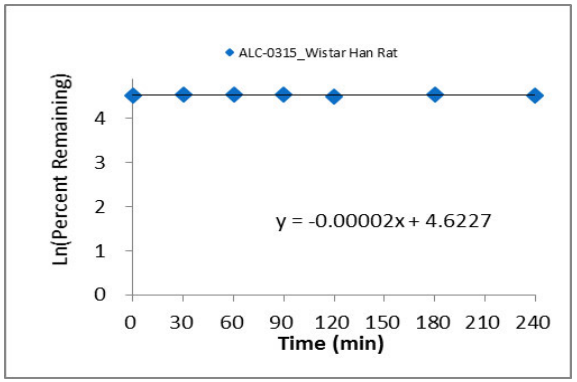
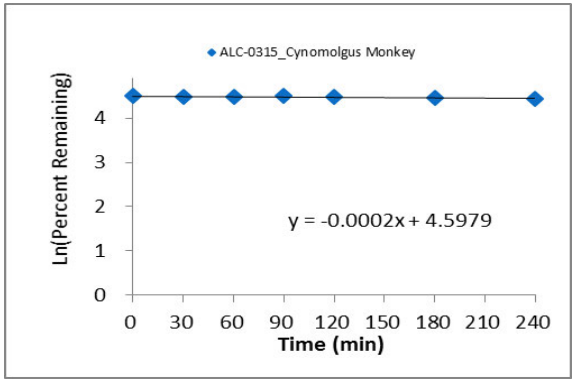
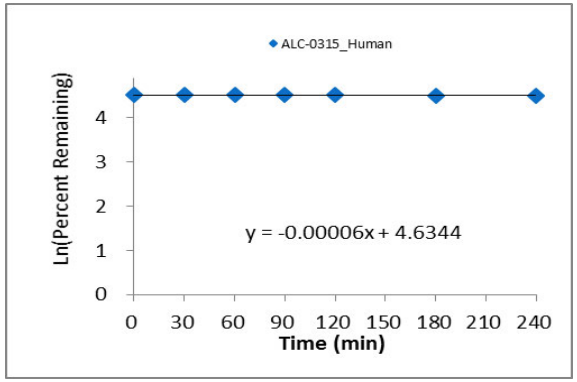
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 2. Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocytes

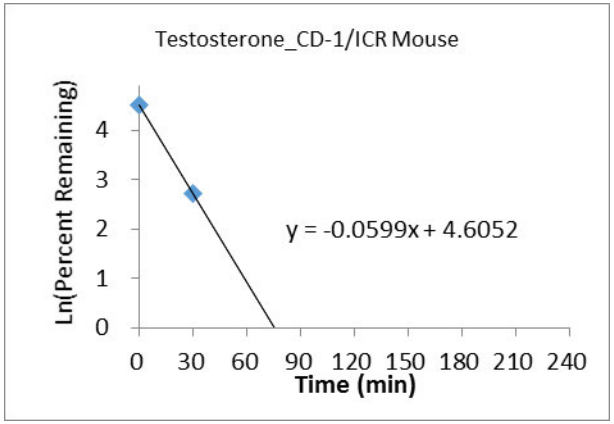
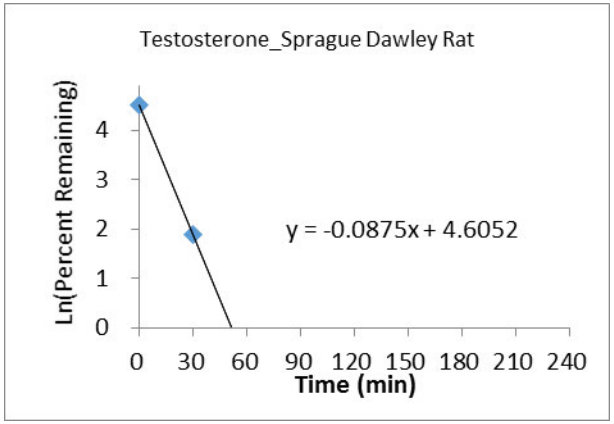
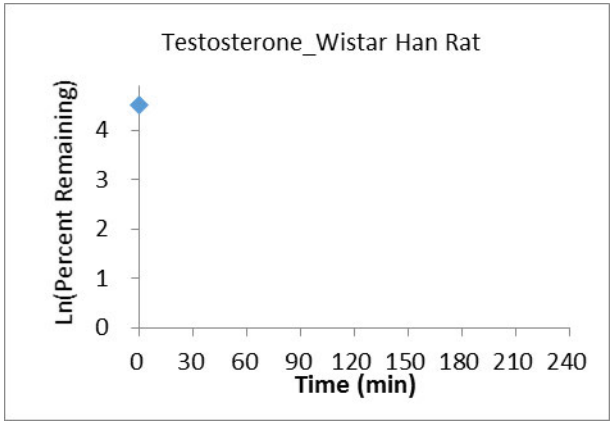
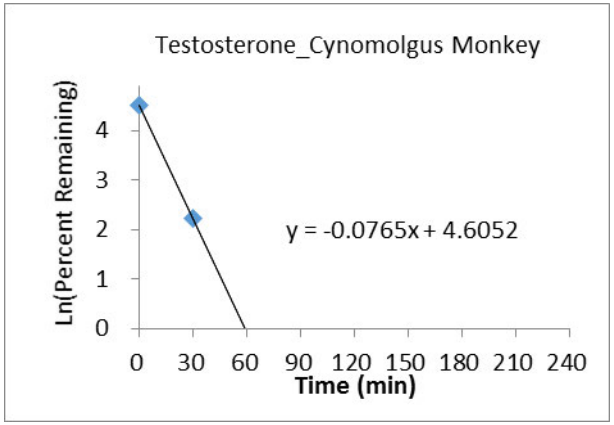
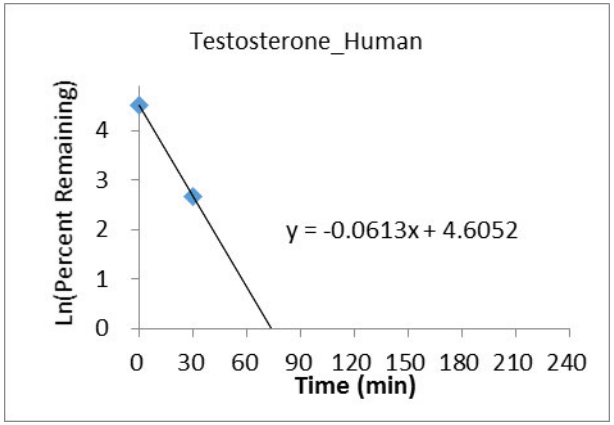
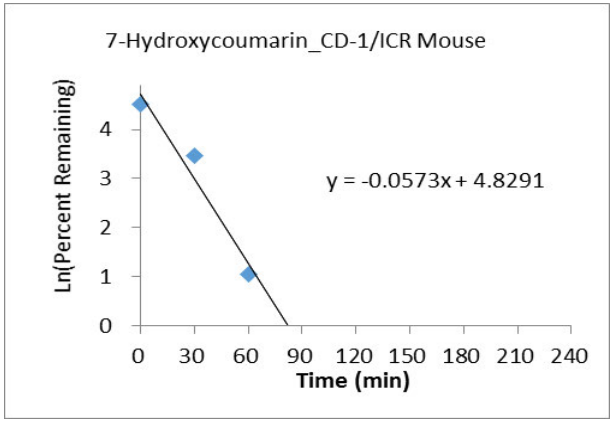
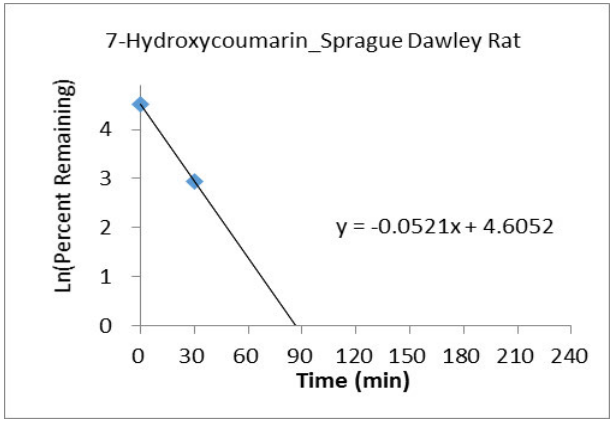
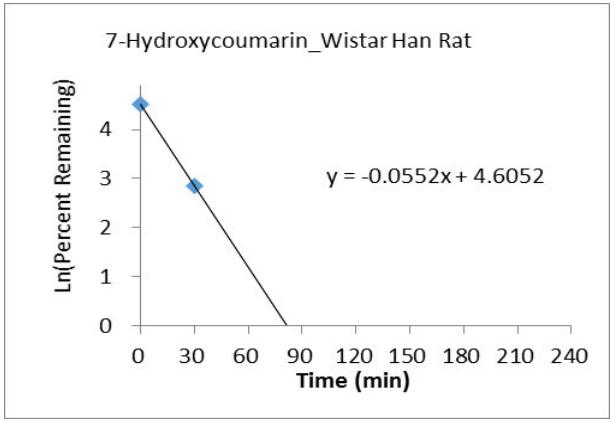
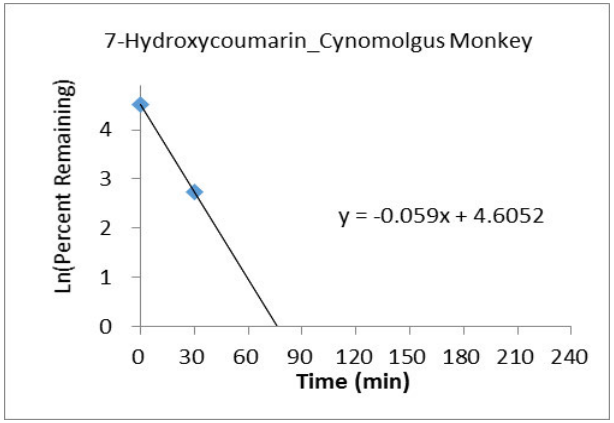
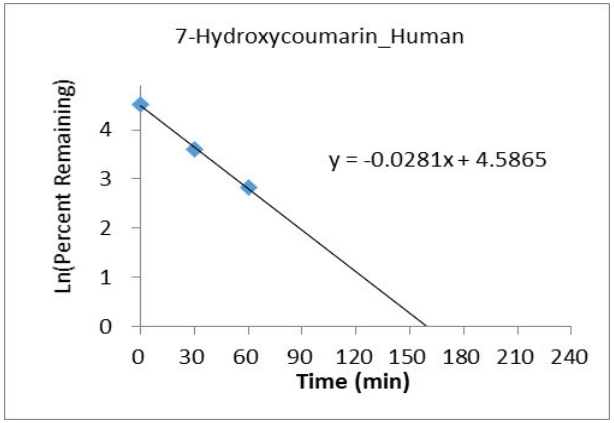
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 3. Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocytes

CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Hepatocytes

Appendix 2 – Stability of ALC-0315 in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

Appendix 3 – Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

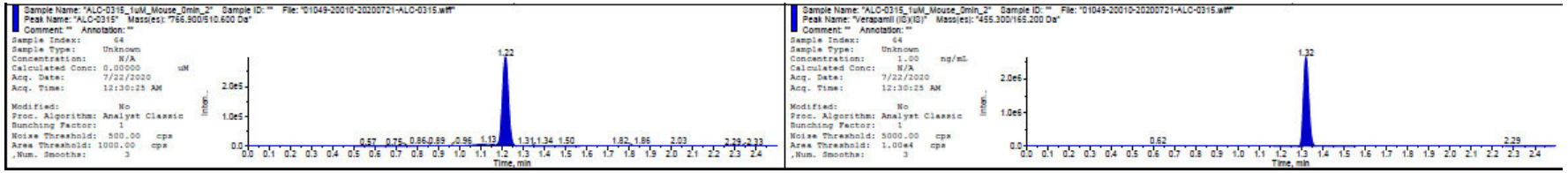
Appendix 4 – Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

Appendix 5 – 01049-20010-ALC-0315-Hepatocytes Stability_Protocol

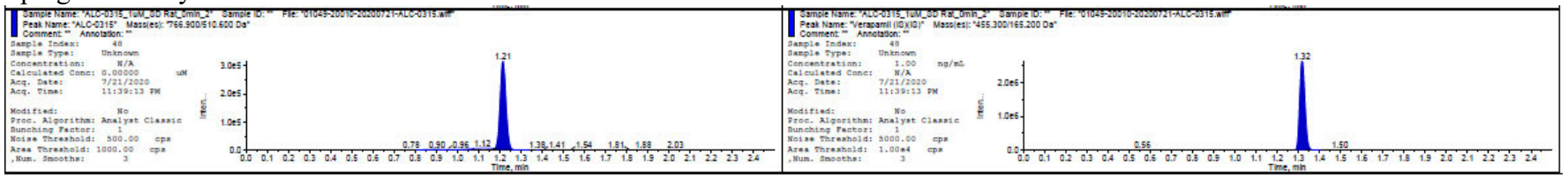
APPENDIX 1

Representative Chromatograms of ALC-0315 in Mouse, Rat, Monkey and Human Hepatocytes

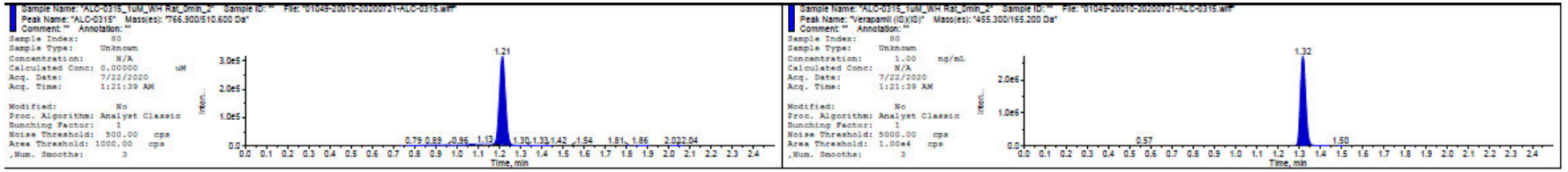
CD 1/ICR mouse



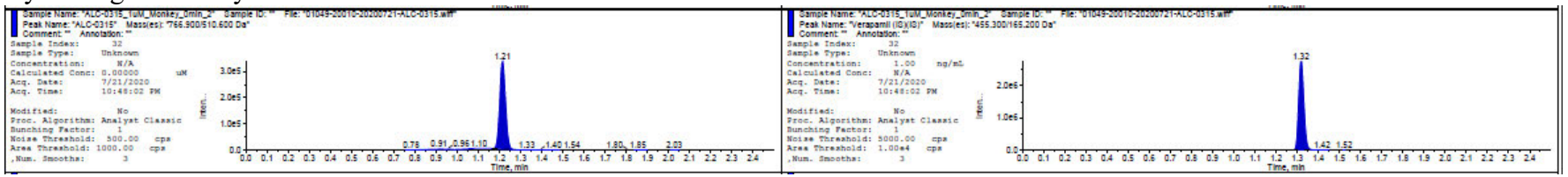
Sprague Dawley rat



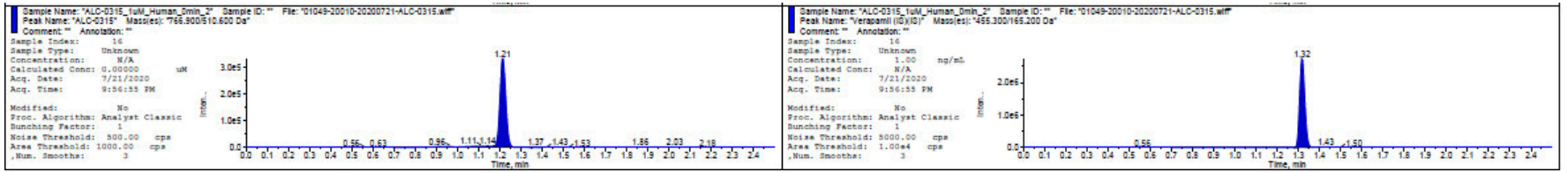
Wistar Han rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0315 in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0315	CD-1/ICR mouse	240	6.00E+05	5.90E+05	4.65E+06	4.50E+06	0.129	0.131
		180	5.99E+05	5.96E+05	4.54E+06	4.53E+06	0.132	0.132
		120	5.86E+05	5.84E+05	4.60E+06	4.45E+06	0.127	0.131
		90	5.88E+05	5.83E+05	4.43E+06	4.38E+06	0.133	0.133
		60	5.83E+05	5.92E+05	4.47E+06	4.47E+06	0.131	0.132
		30	5.94E+05	5.89E+05	4.53E+06	4.43E+06	0.131	0.133
		0	5.92E+05	5.65E+05	4.48E+06	4.39E+06	0.132	0.129
ALC-0315	Sprague Dawley rat	240	6.40E+05	5.94E+05	4.69E+06	4.47E+06	0.137	0.133
		180	6.18E+05	5.83E+05	4.53E+06	4.56E+06	0.136	0.128
		120	5.97E+05	5.77E+05	4.53E+06	4.51E+06	0.132	0.128
		90	6.08E+05	5.81E+05	4.53E+06	4.45E+06	0.134	0.131
		60	6.08E+05	5.75E+05	4.51E+06	4.48E+06	0.135	0.128
		30	6.06E+05	5.67E+05	4.53E+06	4.47E+06	0.134	0.127
		0	6.09E+05	5.82E+05	4.50E+06	4.41E+06	0.135	0.132
ALC-0315	Wistar Han rat	240	5.55E+05	6.12E+05	4.50E+06	4.51E+06	0.123	0.136
		180	5.91E+05	6.06E+05	4.52E+06	4.44E+06	0.131	0.137
		120	5.68E+05	5.83E+05	4.45E+06	4.49E+06	0.128	0.13
		90	5.91E+05	5.94E+05	4.40E+06	4.48E+06	0.134	0.133
		60	5.82E+05	5.99E+05	4.46E+06	4.48E+06	0.131	0.134
		30	6.04E+05	5.94E+05	4.51E+06	4.49E+06	0.134	0.132
		0	5.87E+05	5.88E+05	4.55E+06	4.51E+06	0.129	0.13
ALC-0315	Cynomolgus monkey	240	6.17E+05	5.78E+05	4.65E+06	4.64E+06	0.133	0.125
		180	6.09E+05	5.91E+05	4.59E+06	4.54E+06	0.133	0.13
		120	6.28E+05	5.85E+05	4.61E+06	4.55E+06	0.136	0.129
		90	6.42E+05	6.07E+05	4.55E+06	4.55E+06	0.141	0.134
		60	6.38E+05	5.95E+05	4.66E+06	4.50E+06	0.137	0.132
		30	6.03E+05	6.02E+05	4.61E+06	4.49E+06	0.131	0.134
		0	6.32E+05	6.17E+05	4.54E+06	4.55E+06	0.139	0.136
ALC-0315	Human	240	6.30E+05	6.38E+05	4.52E+06	4.64E+06	0.139	0.138
		180	6.44E+05	6.19E+05	4.66E+06	4.52E+06	0.138	0.137
		120	6.49E+05	9.24E+05	4.65E+06	4.54E+06	0.14	0.204
		90	6.51E+05	6.20E+05	4.60E+06	4.52E+06	0.142	0.137
		60	6.53E+05	6.27E+05	4.54E+06	4.54E+06	0.144	0.138
		30	6.42E+05	6.20E+05	4.54E+06	4.46E+06	0.141	0.139
		0	6.42E+05	6.22E+05	4.56E+06	4.55E+06	0.141	0.137

APPENDIX 3

Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Testosterone	CD-1/ICR mouse	240	LOD	LOD	7.53E+05	7.48E+05	LOD	LOD
		180	LOD	LOD	7.77E+05	7.83E+05	LOD	LOD
		120	LOD	LOD	7.44E+05	7.99E+05	LOD	LOD
		90	LOD	LOD	7.60E+05	7.89E+05	LOD	LOD
		60	LOD	LOD	7.39E+05	7.46E+05	LOD	LOD
		30	5.29E+03	6.16E+03	7.70E+05	7.58E+05	0.007	0.008
		0	3.64E+04	3.41E+04	7.73E+05	7.88E+05	0.047	0.043
Testosterone	Sprague Dawley rat	240	LOD	LOD	8.19E+05	8.01E+05	LOD	LOD
		180	LOD	LOD	7.97E+05	7.54E+05	LOD	LOD
		120	LOD	LOD	7.48E+05	8.25E+05	LOD	LOD
		90	LOD	LOD	8.12E+05	7.45E+05	LOD	LOD
		60	LOD	LOD	7.59E+05	7.44E+05	LOD	LOD
		30	LOD	2.38E+03	8.25E+05	8.19E+05	LOD	0.003
		0	3.38E+04	3.38E+04	8.23E+05	8.59E+05	0.041	0.039
Testosterone	Wistar Han rat	240	LOD	LOD	7.72E+05	8.57E+05	LOD	LOD
		180	LOD	LOD	7.61E+05	7.44E+05	LOD	LOD
		120	LOD	LOD	7.87E+05	7.53E+05	LOD	LOD
		90	LOD	LOD	7.87E+05	7.71E+05	LOD	LOD
		60	LOD	LOD	7.29E+05	7.93E+05	LOD	LOD
		30	LOD	LOD	7.78E+05	7.87E+05	LOD	LOD
		0	3.34E+04	3.39E+04	8.20E+05	7.44E+05	0.041	0.046
Testosterone	Cynomolgus monkey	240	LOD	LOD	8.17E+05	8.22E+05	LOD	LOD
		180	LOD	LOD	8.26E+05	8.16E+05	LOD	LOD
		120	LOD	LOD	8.22E+05	8.12E+05	LOD	LOD
		90	LOD	LOD	8.44E+05	7.91E+05	LOD	LOD
		60	LOD	LOD	8.47E+05	7.85E+05	LOD	LOD
		30	4.32E+03	2.37E+03	8.24E+05	8.22E+05	0.005	0.003
		0	3.45E+04	3.26E+04	8.72E+05	7.93E+05	0.04	0.041
Testosterone	Human	240	LOD	LOD	8.02E+05	8.22E+05	LOD	LOD
		180	LOD	LOD	8.65E+05	8.75E+05	LOD	LOD
		120	LOD	LOD	8.29E+05	8.22E+05	LOD	LOD
		90	LOD	LOD	8.60E+05	8.16E+05	LOD	LOD
		60	LOD	LOD	8.21E+05	8.47E+05	LOD	LOD
		30	6.13E+03	5.10E+03	8.78E+05	8.09E+05	0.007	0.006
		0	3.25E+04	3.56E+04	8.02E+05	8.26E+05	0.04	0.043

LOD = limit of detection

APPENDIX 4

Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
7-Hydroxycoumarin	CD-1/ICR mouse	240	LOD	LOD	6.12E+05	6.29E+05	LOD	LOD
		180	LOD	LOD	6.12E+05	6.09E+05	LOD	LOD
		120	LOD	LOD	6.11E+05	5.99E+05	LOD	LOD
		90	LOD	LOD	6.29E+05	6.06E+05	LOD	LOD
		60	1.33E+03	1.21E+03	6.10E+05	6.25E+05	0.002	0.002
		30	1.25E+04	1.57E+04	6.23E+05	6.31E+05	0.02	0.025
		0	3.97E+04	4.12E+04	6.25E+05	6.37E+05	0.064	0.065
7-Hydroxycoumarin	Sprague Dawley rat	240	LOD	LOD	6.30E+05	6.18E+05	LOD	LOD
		180	LOD	LOD	6.29E+05	6.25E+05	LOD	LOD
		120	LOD	LOD	6.36E+05	6.49E+05	LOD	LOD
		90	LOD	LOD	6.11E+05	6.30E+05	LOD	LOD
		60	LOD	LOD	6.19E+05	6.07E+05	LOD	LOD
		30	8.21E+03	9.55E+03	6.30E+05	6.32E+05	0.013	0.015
		0	3.98E+04	4.10E+04	6.06E+05	5.99E+05	0.066	0.068
7-Hydroxycoumarin	Wistar Han rat	240	LOD	LOD	6.23E+05	6.17E+05	LOD	LOD
		180	LOD	LOD	6.51E+05	6.11E+05	LOD	LOD
		120	LOD	LOD	6.05E+05	6.24E+05	LOD	LOD
		90	LOD	LOD	6.10E+05	6.15E+05	LOD	LOD
		60	LOD	LOD	6.36E+05	6.05E+05	LOD	LOD
		30	6.78E+03	8.59E+03	6.20E+05	6.18E+05	0.011	0.014
		0	4.01E+04	3.94E+04	6.09E+05	6.14E+05	0.066	0.064
7-Hydroxycoumarin	Cynomolgus monkey	240	LOD	LOD	5.82E+05	6.25E+05	LOD	LOD
		180	LOD	LOD	6.01E+05	6.18E+05	LOD	LOD
		120	LOD	LOD	6.38E+05	6.14E+05	LOD	LOD
		90	LOD	LOD	6.38E+05	6.07E+05	LOD	LOD
		60	LOD	LOD	6.28E+05	6.20E+05	LOD	LOD
		30	7.22E+03	6.96E+03	6.42E+05	6.39E+05	0.011	0.011
		0	4.21E+04	4.15E+04	6.44E+05	6.43E+05	0.065	0.065
7-Hydroxycoumarin	Human	240	LOD	LOD	6.04E+05	6.05E+05	LOD	LOD
		180	LOD	LOD	6.45E+05	6.24E+05	LOD	LOD
		120	LOD	LOD	6.28E+05	6.50E+05	LOD	LOD
		90	1.43E+03	1.40E+03	6.42E+05	6.21E+05	0.002	0.002
		60	7.22E+03	8.24E+03	6.20E+05	6.28E+05	0.012	0.013
		30	1.69E+04	1.68E+04	6.27E+05	6.10E+05	0.027	0.028
		0	4.06E+04	3.99E+04	6.01E+05	6.03E+05	0.068	0.066

LOD = limit of detection

APPENDIX 5

01049-20010-ALC-0315-Hepatocytes Stability_Protocol

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road
Pudong, Shanghai 201299
China

Study Number
01049-20010

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20010

1.2. Study Title

In Vitro Metabolic Stability of ALC-0315 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

1.3. Sponsor Representative

(b) (6)

Acuitas Therapeutics Inc.
6190 Agronomy Road, Suite 402
Vancouver BC V6T 1Z3
Canada

(b) (6)

1.4. Objective

To evaluate the *in vitro* metabolic stability of ALC-0315 in Hepatocytes from different species and to determine intrinsic clearance in each species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

2. MATERIALS

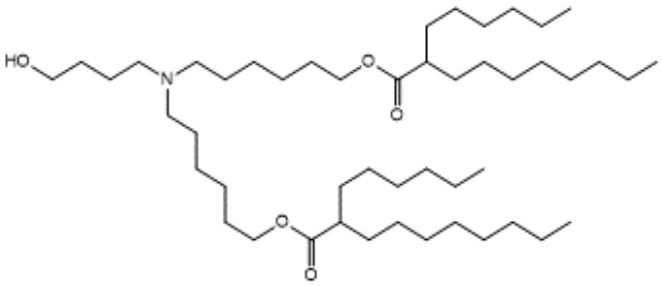
2.1. Test Article

Name: ALC-0315

Molecular Formula: $C_{48}H_{95}NO_5$

MW (g/mol): 766.27

Exact Mass: 765.72



2.2. Positive Control and Internal Standard

Testosterone and 7-hydroxycoumarin will be used as positive controls. Verapamil will be used as internal standard. The sources will be documented in the experimental records and presented in the report.

2.3. Hepatocytes

Cryopreserved hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in liquid nitrogen until use. The source(s) and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solution: Appropriate amount of test article or positive control is weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain a 10 mM stock solution.

(2) Preparation of 4 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of Stock Solution (mM)	Volume of Stock Solution (μL)	Volume of DMSO (μL)	Final Concentration (mM)
10	20	30	4

(3) Preparation of 2 μM dosing solution(2×) of test article or positive control:

Dosing Solution (2×) of Test Article or Positive Control			
Conc. of Spiking Solution (mM)	Volume of Spiking Solution (μL)	Volume of William's E Medium (μL)	Final Concentration (μM)
4	2	3998	2

(4) Preparation of hepatocyte suspension: Thaw cryopreserved hepatocytes in a 37℃ water bath. Transfer the hepatocytes to hepatocyte thawing medium (William's E Medium with 30% percoll and 5% FBS) and centrifuge at 100×g for 10 min at room temperature. Resuspend the cell pellet with William's E Medium and determine cell viability by trypan blue exclusion analysis and calculate the viable cell density. Dilute the hepatocytes with incubation medium to an appropriate density (2×10^6 viable cells/mL) and pre-warm at 37 °C for 10 min.

(5) 40 μL of each hepatocyte suspension is added to 96-well plates in duplicate for each time point (0, 30, 60, 90, 120, 180, and 240 min).

(6) For 0 min samples: 480 μL of internal standard solution (IS solution, 10 ng/mL verapamil in ethanol) is added, followed by 40 μL of pre-warmed 2× dosing solution. The final concentration of test article or positive control in the incubation mixture is 1 μM.

(7) For the 30, 60, 90, 120, 180, and 240 min samples, 40 μL of pre-warmed 2× dosing solution is added to initiate reaction. The final concentration of test article or positive control in the incubation mixture is 1 μM.

(8) The samples are incubated at 37℃ . At 30, 60, 90, 120, 180, and 240 min time points, stop the reaction by adding 480 μL ethanol containing internal standard to all of the duplicate wells.

(9) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(10) The plates are sealed and stored at -20℃ freezer until bioanalysis.

(11) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 μL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

Waters Acquity UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column：ACQUITY UPLC BEH HILIC 1.7μm (2.1*100mm)

Gradient for ALC-0315:

Time (min)	Solvent A (%)	Solvent B (%)
0.01	5	95
1.50	40	60
2.00	40	60
2.01	5	95
2.50	5	95

A: 10mM ammonium formate, 0.1% Formic acid in water

B: 10mM ammonium formate, 0.1% Formic acid in acetonitrile

Flow rate：500 μL/min

Column temperature：40 ºC

Autosampler temperature: 4ºC

Compound	Q1(m/z)	Q3(m/z)	Retention Time (min)
ALC-0315	766.90	510.60	~1.07
Verapamil (IS)	455.30	165.20	~1.19

5. DATA ANALYSIS

The % remaining (parent compound) will be calculated by dividing the peak area ratio (compound peak area/ internal standard peak area) by the 0 min peak area ratio. The natural logarithm of % remaining is plotted against time and the slope of the fitted line will be determined as follows:

Elimination rate constant (k) = - slope

Half-life ($T_{1/2}$) (minutes) = 0.693/k

Intrinsic clearance predicted from the *in vitro* hepatocyte stability study will be calculated as shown below:

CL'_{int} (mL/min/kg) = k * V (1 mL incubation/10^6 cells) * Scaling Factor (10^6 cells/kg),

Scaling Factor (10^6 cells/kg) = Hepatocellularity (10^6 cells/g liver) * Normalized Liver Weight (g liver/kg body weight)

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Hepatocytes

Species	Hepatocellularity (10^6 cells/g liver)	Liver Weight (g/kg BW)	Scaling Factor (10^6 cells/kg)
Mouse	135	87.5	11812.5
Rat	117	40	4680
Monkey	120	32	3840
Human	99	25.7	2544.3

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

Sponsor Approval

(b) (6)

Sponsor Representative

July 15, 2020

Date

Study Director Approval

(b) (6)

Study Director

2020/07/15

Date

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20020
Experimental Start Date	2020-06-04
Experimental Completion Date	2020-06-08
Number of Pages in Report	28

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SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0159 in liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 2-hour incubation with liver microsomes from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6)

Study Director

2020/08/04
Date

Sponsor Approval:

(b) (6)

Study Monitor

August 4, 2020
Date

1. OBJECTIVE

To evaluate the in vitro metabolic stability of ALC-0159 in liver microsomes from different species.

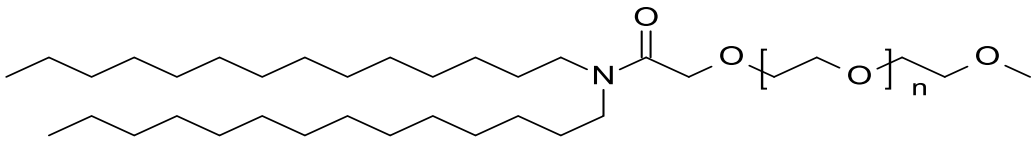
2. MATERIALS

2.1 Test Article

Name: ALC-0159

Molecular Formula: $C_{30}H_{60}NO\ (C_2H_4O)_nOCH_3$ n = 45-50

MW (g/mol): ~2400-2600



2.2 Positive Control

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Ketanserin	TCI	74050-98-9	K0051	NPGAF-CO	395.43

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Tolbutamide	Sigma-Aldrich	64-7-7	46968	BCBV8457	270.35

2.4 Liver Microsomes and Cofactor

The following pooled liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were stored in a -70°C ultra low temperature freezer prior to use.

Species	Manufacturer	Cat. No.	Lot No.	Protein Concentration (mg/mL)
CD-1/ICR mouse (male)	XenoTech	M1000	1910002	20
Sprague Dawley rat (male)	XenoTech	R1000	1910100	20
Wistar Han rat	BioIVT	BCF	201801BCF	20
Cynomolgus monkey (male)	RILD Shanghai	LM-SXH-02M	NXNN	20
Human (mixed gender)	XenoTech	H0610	1810003	20

2.5 Coenzyme

NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt was stored at 2-8°C in a refrigerator prior to use.

Compound Name	Manufacturer	Cat. No.	Molecular Weight	Purity
NADPH	Roche Diagnostic	10621706001	833.35	97%

3. EXPERIMENTAL PROCEDURES

3.1 Stock solution: 1.90 mg of ALC-0159 was weighed and dissolved in 76 µL of DMSO to obtain a 10 mM stock solution. 3.237 mg of ketanserin was weighed and dissolved in 818.60 µL of DMSO to obtain a 10 mM stock solution.

3.2 0.5 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution (mM)	Volume of stock solution (µL)	Volume of MeOH (µL)	Final Concentration (mM)
10	10	190	0.5

3.3 1.5× liver microsomes suspension containing test article or positive control:

1.5× Liver Microsomes Suspension Containing Test Article or Positive Control					
Liver Microsomes		0.5 mM spiking solution (µL)	100 mM potassium phosphate buffer (pH 7.4) (µL)	Final Concentration	
Conc. of stock suspension (mg/mL)	Volume of stock suspension (µL)			Liver microsomal protein (mg/mL)	Compound (µM)
20	18.75	1.5	479.75	0.75	1.5

3.4 22.98 mg of NADPH was weighed and dissolved in 4.596 mL of 100 mM potassium phosphate buffer to obtain a 6 mM NADPH working solution. This working solution was then pre-warmed at 37ºC.

3.5 30 µL of 1.5× liver microsomes suspension containing test article or positive control was added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

3.6 96-well incubation plates were pre-warmed at 37 ºC for 5 min.

3.7 For 0-min samples: 450 µL of ethanol containing internal standard (IS solution) was added before 15 µL of pre-warmed NADPH working solution (6 mM) was added.

3.8 For other samples (15, 30, 60, 90, and 120 min): 15 µL of pre-warmed NADPH working solution (6 mM) was added to initiate the reaction.

Volume (µL)			Final Concentration in Incubation Mixture		
1.5× Liver Microsomes Suspension Containing Test Article or Positive Control	3×NADPH working solution	Total	Liver microsomal protein (mg/mL)	Test Article or Positive Control (µM)	NADPH (mM)
30	15	45	0.5	1	2

The samples were incubated at 37 ºC and 450 µL of IS solution was added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

3.9 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuged at 6,000 rpm for 15 min.

3.10 200 µL of supernatant was transferred from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1 Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column：Agilent Zorbax SB-CN 3.5um (100mm*2.1mm)

Gradient for ALC-0159:

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

Solvent A: 0.1% formic acid in water

Solvent B: 0.1% formic acid in acetonitrile

Flow rate：600 μL/min

Column temperature：40 °C

Autosampler temperature: 4°C

MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time
ALC-0159	1164.00	494.70	45	71	~1.31
Tolbutamide(IS)	271.10	172.00	70	18	~1.01

4.3 Detection of ALC-0159

Representative chromatograms of ALC-0159 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0159 or positive control, ketanserin) was calculated by dividing the peak area ratio (test article peak area/internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life was calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope

Half-life ($t_{1/2}$) = 0.693/k

The *in vitro* intrinsic clearance, CL'_{int}, was calculated from the $t_{1/2}$ as follows:

CL'_{int} = (0.693/$t_{1/2}$) × (1/ (microsomal protein concentration (0.5 mg/mL))) × Scaling Factor

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Liver Microsomes

Species	Microsomal Protein (mg) per Gram of Liver	Liver Weight (g) per kg Body Weight	Scaling Factor (mg/kg)[a]	Hepatic Blood Flow (mL/min/kg)
Mouse	45	87.5	3937.5	90
Rat	44.8	40	1792	55.2
Monkey	45	32.5	1462.5	44
Human	48.8	25.7	1254.2	20.7

[a]Microsomal protein (mg/g liver) × liver weight (g)/kg body weight

6. RESULTS

A summary of the % remaining parent compound, CL'_{int} and half-life of ALC-0159 obtained from a 2-hour incubation of ALC-0159 with liver microsomes from CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human is presented in Table 2. The stability of ALC-0159 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The liver microsomes used in this study were tested for activity using a metabolism control substrate under incubation conditions identical to those used for ALC-0159. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compound (ketanserin) during the 2-hour incubation period, hence the test systems were considered to have yielded valid results. A summary of the % remaining parent compound, CL'_{int} and half-life of ketanserin is provided in Table 2. The stability of ketanserin over time in each matrix is shown in Figure 2. Raw data is presented in Appendix 3.

7. CONCLUSIONS

This study evaluated the *in vitro* metabolic stability of ALC-0159 in liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 2-hour incubation with liver microsomes from all these species.

Table 2. Summary of Liver Microsomal Stability of ALC-0159 and Ketanserin

Test Article	Species		Percent Remaining (%)						$t_{1/2}$ (minute)	CL'int (mL/min/kg)
			0 min	15 min	30 min	60 min	90 min	120 min		
ALC-0159	CD-1/ICR mouse	Mean	100.00	82.27	86.40	85.54	85.41	95.87	**>120**	<45.5
		RSD of Area Ratio	0.07	0.09	0.11	0.01	0.05	0.18		
	Sprague Dawley rat	Mean	100.00	101.24	93.78	98.34	95.44	97.10	**>120**	<20.7
		RSD of Area Ratio	0.09	0.03	0.08	0.03	0.05	0.11		
	Wistar Han rat	Mean	100.00	112.11	102.69	105.38	100.90	108.97	**>120**	<20.7
		RSD of Area Ratio	0.01	0.06	0.06	0.01	0.04	0.13		
	Cynomolgus monkey	Mean	100.00	100.83	85.12	86.36	94.63	93.39	**>120**	<16.9
		RSD of Area Ratio	0.06	0.07	0.03	0.03	0.04	0.05		
	Human	Mean	100.00	99.59	92.28	95.53	97.97	93.09	**>120**	<14.5
		RSD of Area Ratio	0.01	0.11	0.03	0.05	0.02	0.02		
Ketanserin	CD-1/ICR mouse	Mean	100.00	61.73	37.16	17.24*	10.16*	6.43*	**21.0**	260
		RSD of Area Ratio	0.04	0.01	0.02	0.05	0.01	0.05		
	Sprague Dawley rat	Mean	100.00	74.03	51.43	26.11	16.08*	10.01*	**30.7**	80.9
		RSD of Area Ratio	0.04	0.02	0.03	0.05	0.03	0.03		
	Wistar Han rat	Mean	100.00	54.03	25.10	6.76	2.35	1.18*	**16.4**	151
		RSD of Area Ratio	0.02	0.02	0.01	0.07	0.04	0.06		
	Cynomolgus monkey	Mean	100.00	71.44	47.42	24.00	13.05*	8.35*	**28.9**	70.1
		RSD of Area Ratio	0.03	0.02	0.01	0.02	0.04	0.02		
	Human	Mean	100.00	77.74	57.56	38.26	26.22*	24.46*	**43.1**	40.3
		RSD of Area Ratio	0.09	0.01	0.01	0.04	0.12	0.05		

* Compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked in * were in the slow disappearance phase and were excluded from half-life calculation.

Figure 1. Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes

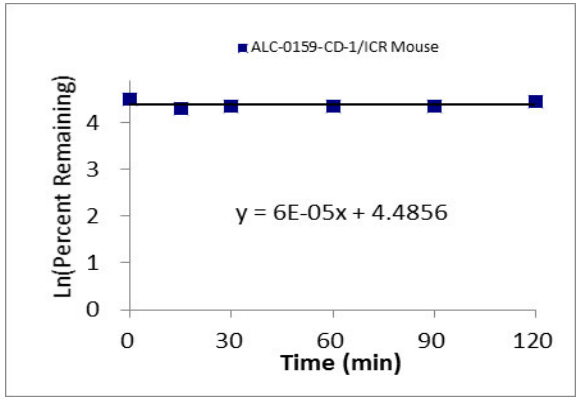
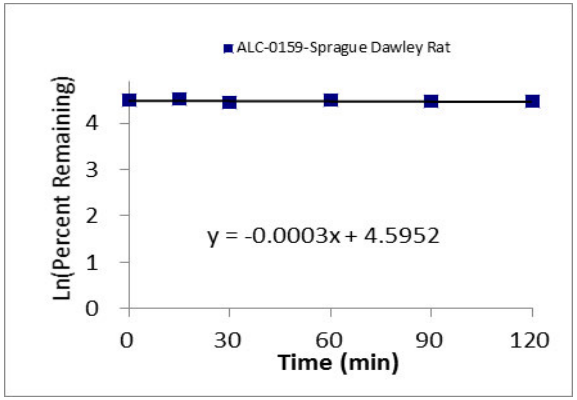
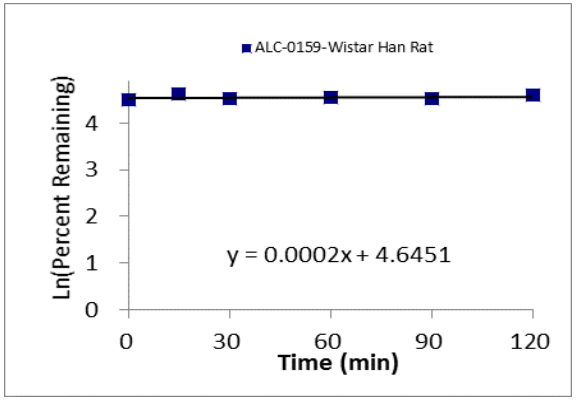
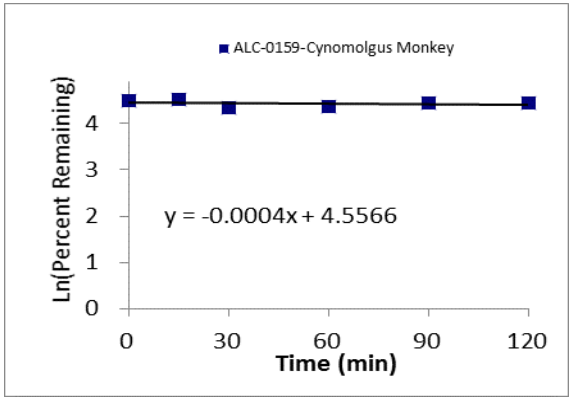
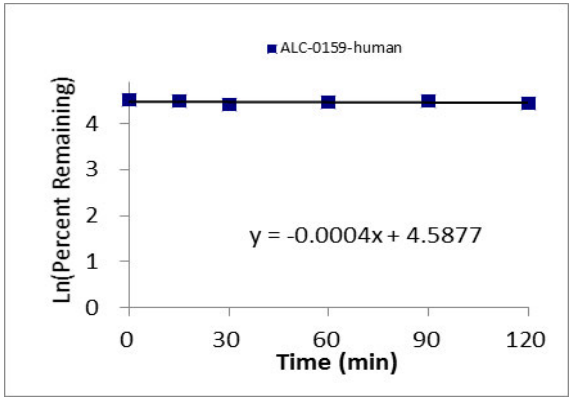
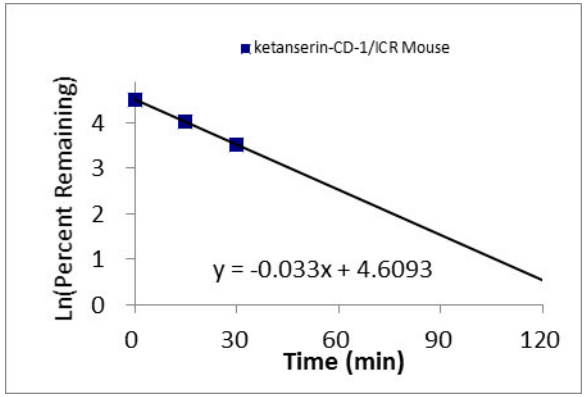
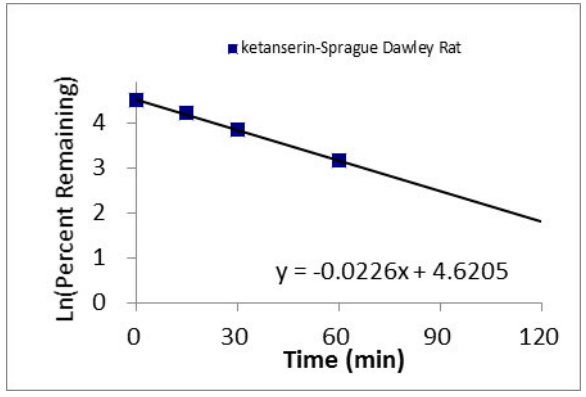
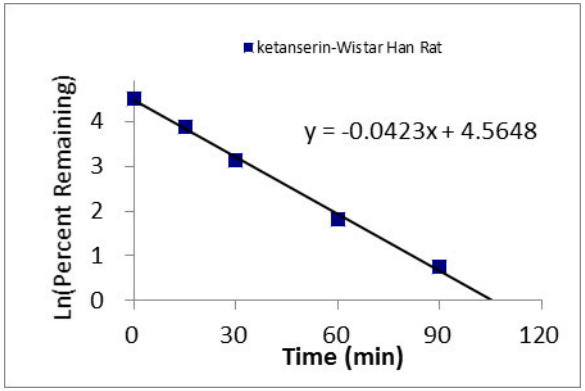
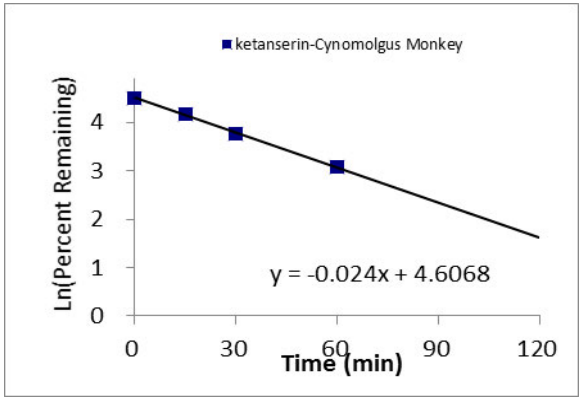
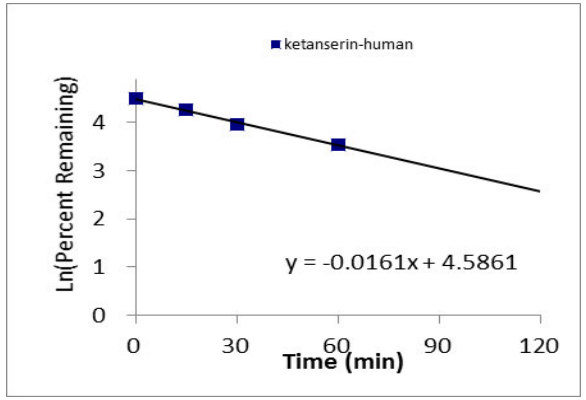
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 2. Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes

CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes

Appendix 2 – Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

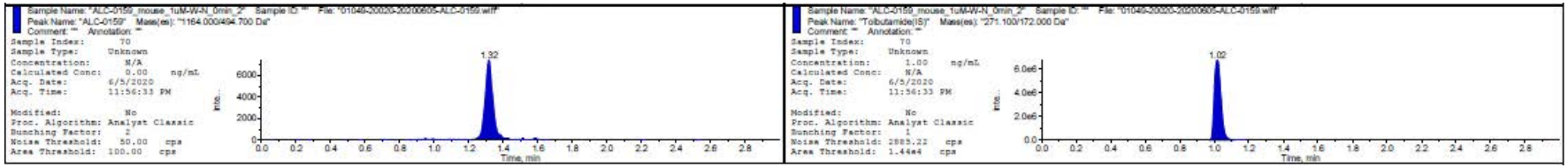
Appendix 3 – Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Appendix 4 –01049-20020-microsomal stability protocol

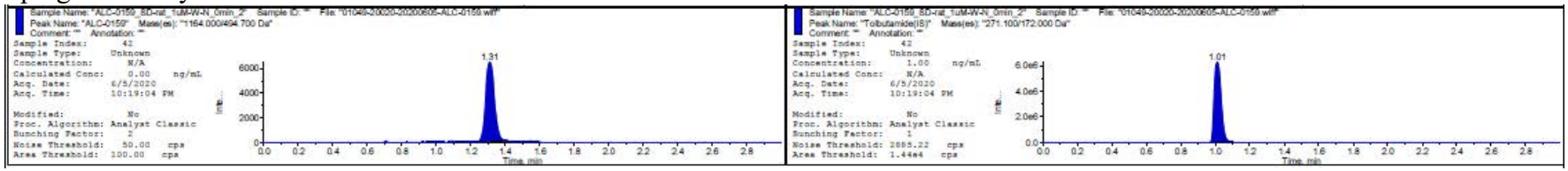
APPENDIX 1

Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes

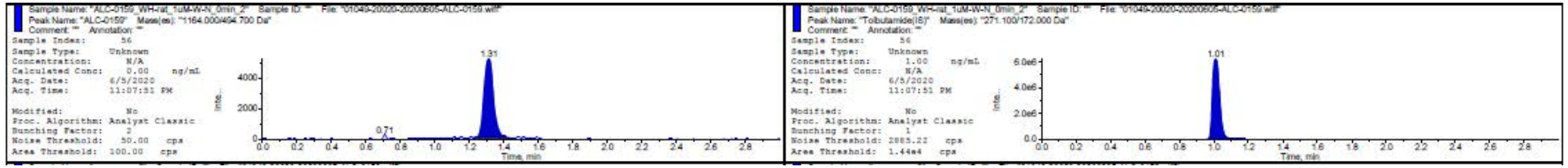
CD-1/ICR mouse



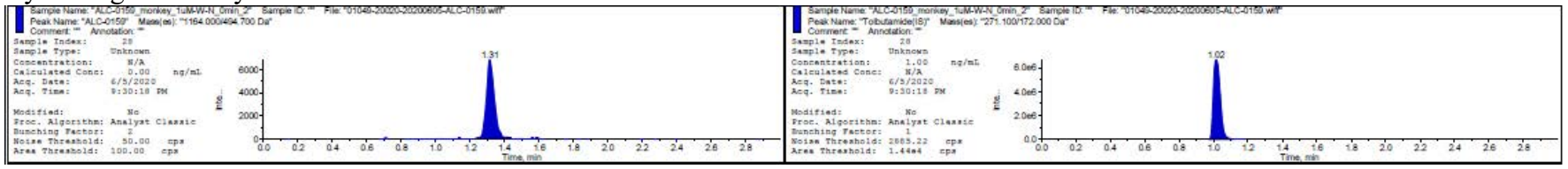
Sprague Dawley rat



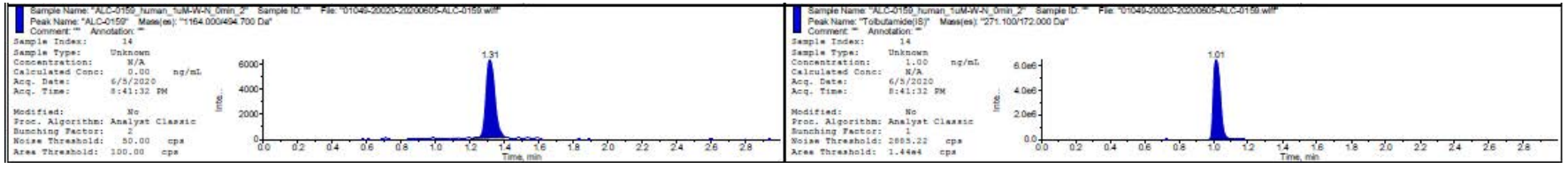
Wistar Han rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0159	CD-1/ICR mouse	0	2.04E+04	2.24E+04	1.77E+07	1.77E+07	0.001	0.001
		15	1.43E+04	1.88E+04	1.54E+07	1.78E+07	0.001	0.001
		30	2.04E+04	1.50E+04	1.80E+07	1.56E+07	0.001	0.001
		60	1.88E+04	1.85E+04	1.83E+07	1.79E+07	0.001	0.001
		90	1.80E+04	1.90E+04	1.81E+07	1.78E+07	0.001	0.001
		120	1.88E+04	2.31E+04	1.86E+07	1.77E+07	0.001	0.001
ALC-0159	Sprague Dawley rat	0	2.03E+04	2.23E+04	1.79E+07	1.74E+07	0.001	0.001
		15	2.12E+04	2.24E+04	1.79E+07	1.78E+07	0.001	0.001
		30	1.93E+04	2.16E+04	1.81E+07	1.81E+07	0.001	0.001
		60	2.01E+04	2.11E+04	1.74E+07	1.75E+07	0.001	0.001
		90	1.97E+04	2.08E+04	1.78E+07	1.75E+07	0.001	0.001
		120	1.95E+04	2.17E+04	1.81E+07	1.72E+07	0.001	0.001
ALC-0159	Wistar Han rat	0	1.97E+04	1.98E+04	1.78E+07	1.76E+07	0.001	0.001
		15	2.27E+04	2.13E+04	1.75E+07	1.77E+07	0.001	0.001
		30	2.00E+04	2.15E+04	1.82E+07	1.81E+07	0.001	0.001
		60	2.06E+04	2.09E+04	1.77E+07	1.77E+07	0.001	0.001
		90	1.96E+04	1.94E+04	1.70E+07	1.78E+07	0.001	0.001
		120	2.27E+04	1.89E+04	1.71E+07	1.72E+07	0.001	0.001
ALC-0159	Cynomolgus monkey	0	2.31E+04	2.12E+04	1.83E+07	1.83E+07	0.001	0.001
		15	2.14E+04	2.36E+04	1.84E+07	1.85E+07	0.001	0.001
		30	2.00E+04	1.91E+04	1.91E+07	1.90E+07	0.001	0.001
		60	1.90E+04	2.03E+04	1.86E+07	1.89E+07	0.001	0.001
		90	2.08E+04	2.14E+04	1.88E+07	1.82E+07	0.001	0.001
		120	2.04E+04	2.18E+04	1.87E+07	1.86E+07	0.001	0.001
ALC-0159	Human	0	2.23E+04	2.15E+04	1.80E+07	1.76E+07	0.001	0.001
		15	2.30E+04	2.02E+04	1.74E+07	1.79E+07	0.001	0.001
		30	2.08E+04	2.02E+04	1.80E+07	1.82E+07	0.001	0.001
		60	2.03E+04	2.13E+04	1.80E+07	1.75E+07	0.001	0.001
		90	2.14E+04	2.10E+04	1.75E+07	1.76E+07	0.001	0.001
		120	2.01E+04	2.01E+04	1.77E+07	1.74E+07	0.001	0.001

APPENDIX 3

Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Stability of Ketanserin in Mouse, Rat, Monkey and Human Liver Microsomes – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Ketanserin	CD-1/ICR mouse	0	1.93E+06	1.99E+06	8.68E+05	8.45E+05	2.22	2.36
		15	1.18E+06	1.17E+06	8.32E+05	8.31E+05	1.42	1.41
		30	7.30E+05	7.08E+05	8.43E+05	8.45E+05	0.87	0.84
		60	3.42E+05	3.24E+05	8.37E+05	8.49E+05	0.41	0.38
		90	1.94E+05	1.94E+05	8.29E+05	8.36E+05	0.23	0.23
		120	1.20E+05	1.28E+05	8.43E+05	8.39E+05	0.14	0.15
Ketanserin	Sprague Dawley rat	0	2.00E+06	1.93E+06	8.58E+05	8.74E+05	2.33	2.21
		15	1.42E+06	1.46E+06	8.57E+05	8.57E+05	1.66	1.70
		30	9.99E+05	1.00E+06	8.34E+05	8.78E+05	1.20	1.14
		60	5.01E+05	5.15E+05	8.76E+05	8.37E+05	0.57	0.61
		90	3.16E+05	3.07E+05	8.43E+05	8.62E+05	0.37	0.36
		120	1.91E+05	1.89E+05	8.55E+05	8.14E+05	0.22	0.23
Ketanserin	Wistar Han rat	0	2.02E+06	2.08E+06	8.55E+05	8.52E+05	2.36	2.44
		15	1.08E+06	1.09E+06	8.41E+05	8.28E+05	1.28	1.31
		30	5.31E+05	5.23E+05	8.76E+05	8.71E+05	0.61	0.60
		60	1.29E+05	1.41E+05	8.41E+05	8.24E+05	0.15	0.17
		90	4.80E+04	4.97E+04	8.74E+05	8.55E+05	0.05	0.06
		120	2.31E+04	2.42E+04	8.56E+05	8.22E+05	0.03	0.03
Ketanserin	Cynomolgus monkey	0	2.07E+06	2.07E+06	8.64E+05	8.34E+05	2.40	2.49
		15	1.43E+06	1.46E+06	8.30E+05	8.23E+05	1.72	1.77
		30	9.68E+05	9.82E+05	8.42E+05	8.42E+05	1.15	1.17
		60	4.84E+05	4.88E+05	8.40E+05	8.18E+05	0.58	0.60
		90	2.68E+05	2.75E+05	8.65E+05	8.40E+05	0.31	0.33
		120	1.69E+05	1.65E+05	8.19E+05	8.19E+05	0.21	0.20
Ketanserin	Human	0	2.11E+06	1.97E+06	8.12E+05	8.57E+05	2.60	2.30
		15	1.57E+06	1.56E+06	8.30E+05	8.13E+05	1.89	1.92
		30	1.09E+06	1.19E+06	7.77E+05	8.37E+05	1.40	1.42
		60	7.23E+05	6.78E+05	7.52E+05	7.42E+05	0.96	0.91
		90	6.14E+05	5.18E+05	8.82E+05	8.80E+05	0.70	0.59
		120	4.40E+05	4.88E+05	7.60E+05	7.88E+05	0.58	0.62

APPENDIX 4

01049-20020-microsomal stability protocol

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road
Pudong, Shanghai 201299
China

Study Number
01049-20020

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20020

1.2. Study Title

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Liver Microsomes

1.3. Sponsor Representative

(b) (6)
Acuitas Therapeutics Inc.
6190 Agronomy Road, Suite 402
Vancouver BC V6T 1Z3
Canada
(b) (6)

1.4. Objective

To evaluate the *in vitro* metabolic stability of ALC-0159 in liver microsomes from different species and to determine intrinsic clearance in each species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

(b) (6)

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

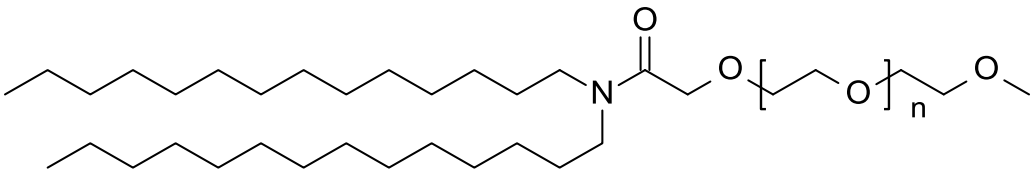
2. MATERIALS

2.1. Test Article

Name: ALC-0159

Molecular Formula: $C_{30}H_{60}NO\ (C_2H_4O)_n$ (n = 45~50)

MW (g/mol): ~2400-2600



2.2. Positive Control and Internal Standard

Ketanserin and verapamil will be used as positive control and internal standard, respectively. The sources will be documented in experimental records and presented in the report.

2.3. Liver Microsomes and Cofactor

Liver microsomes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in a -70ºC ultra low temperature freezer. NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt were purchased from a qualified supplier and stored at 2-8ºC in a refrigerator. The source and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solution: Appropriate amount of test article or positive control is weighed and dissolved in DMSO to obtain a 10 mM stock solution.

(2) Preparation of 0.5 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution (mM)	Volume of stock solution (μL)	Volume of MeOH (μL)	Final Concentration (mM)
10	10	190	0.5

(3) Preparation of 1.5× liver microsomes suspension containing test article or positive control:

1.5× Liver Microsomes Suspension Containing Test Article or Positive Control					
Liver Microsomes		0.5 mM spiking solution (μL)	100 mM potassium phosphate buffer (pH 7.4) (μL)	Final Concentration	
Conc. of stock suspension (mg/mL)	Volume of stock suspension (μL)			Liver microsomal protein (mg/mL)	Compound (μM)
20	18.75	1.5	479.75	0.75	1.5

(4) 3×NADPH working solution (6 mM; 5 mg/mL) will be prepared by dissolving NADPH in 100 mM pH 7.4 potassium phosphate buffer. The working solution is then pre-warmed at 37ºC.

(5) 30 μL of 1.5× liver microsomes suspension containing test article or positive control is added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

(6) 96-well incubation plates are pre-warmed at 37 ºC for 5 min.

(7) For 0-min samples: 450 μL ethanol containing internal standard (IS solution) is added before 15 μL pre-warmed NADPH working solution (6mM) is added.

(8) For other samples (15, 30, 60, 90, and 120 min): 15 μL pre-warmed NADPH working solution (6 mM) is added to initiate reaction.

Volume (μL)			Final Concentration in incubation mixture		
1.5× Liver Microsomes Suspension Containing Test Article or Positive Control	3×NADPH working solution	Total	Liver microsomal protein (mg/mL)	Test Article or Positive Control (μM)	NADPH (mM)
30	15	45	0.5	1	2

The samples are incubated at 37 ºC and 450 µL IS solution is added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

(9) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(10) The plates are sealed and stored at -20 ºC in a freezer until bioanalysis.

(11) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 µL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column: Agilent Zorbax SB-CN 3.5um (100mm*2.1mm)

Gradient for ALC-0159

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

A: 0.1%Formic acid in water

B: 0.1%Formic acid in acetonitrile

Flow rate：600 µL/min

Column temperature：40 ºC

Autosampler temperature: 4ºC

Compound	Q1(m/z)	Q3(m/z)	Retention Time (min)
ALC-0159	1164.00	494.70	~1.30
Tolbutamide (IS)	271.10	172.00	~1.02

5. TA ANALYSIS

The % remaining will be calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining will be plotted against time, and the slope of the regression line will be determined. Then elimination constant and half-life will be calculated as below.

$$\text{Elimination rate constant (k)} = - \text{ slope}$$

$$\text{Half-life } (t_{1/2}) = 0.693/k$$

The *in vitro* intrinsic clearance, CL'_{int}, will be calculated from the $t_{1/2}$ as follows:

$$CL'_{int} = (0.693/T_{1/2}) \times (1/(\text{microsomal protein concentration (0.5 mg/mL)})) \times \text{Scaling Factor}$$

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Liver Microsomes

Species	Microsomal Protein (mg) per Gram of Liver	Liver Weight (g) per kg Body Weight	Scaling Factor (mg/kg)[a]	Hepatic Blood Flow (mL/min/kg)
Mouse	45	87.5	3937.5	90
Rat	44.8	40	1792	55.2
Monkey	45	32.5	1462.5	44
Human	48.8	25.7	1254.2	20.7

[a]Microsomal protein (mg/g liver) × liver weight (g)/kg body weight

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

Sponsor Approval

(b) (6)

June 3, 2020

Date

Sponsor Representative

Study Director Approval

(b) (6)

2020/06/03

Date

Study Director

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20021
Experimental Start Date	2020-06-19
Experimental Completion Date	2020-06-24
Number of Pages in Report	31

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SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0159 in liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 2-hour incubation with liver S9 from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6)

Study Director

2020/8/10

Date

Sponsor Approval:

(b) (6)

Study Monitor

August 10, 2020

Date

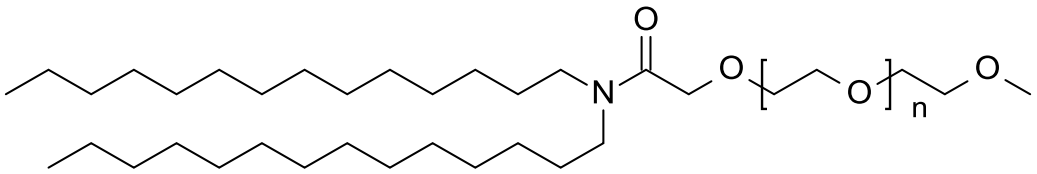
1. OBJECTIVE

To evaluate the *in vitro* metabolic stability of ALC-0159 in liver S9 fractions from different species.

2. MATERIALS

2.1 Test Article

Name: ALC-0159
Molecular Formula: C30H60NO (C2H4O) n (n = 45~50)
MW (g/mol): ~2400-2600



2.2 Positive Controls

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Testosterone	Aladdin	58-22-0	T102169	K1505051	288.42
7-hydroxycoumarin	J&K Scientific	93-35-6	153384	L630I04	162.14

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Verapamil hydrochloride	TCI	152-11-4	V0118	RFMWJ-RL	491.06
Tolbutamide	Sigma-Aldrich	64-7-7	46968	BCBV8457	270.35

2.4 Liver S9 Fractions

The following pooled liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human were stored in a -70°C ultra low temperature freezer prior to use.

Species	Manufacturer	Cat. No.	Lot No.	Protein Concentration (mg/mL)
CD-1/ICR mouse (male)	XenoTech	M1000.S9	1310210	20
Sprague Dawley rat (male)	XenoTech	R1000.S9	1310212	20
Cynomolgus monkey (male)	XenoTech	P2000.S9	0910273	20
Human (mixed gender)	BioreclamationIVT	X008023	BKY	20

2.5 Coenzymes and Pore-forming Agent

NADPH (reduced β-nicotinamide adenine dinucleotide 2′-phosphate) tetrasodium salt was stored at 2~8ºC in a refrigerator prior to use. UDPGA (uridine-diphosphate-glucuronic acid trisodium salt) and alamethicin were stored in a -20ºC freezer prior to use.

Compound Name	Manufacturer	Cat. No.	Molecular Weight	Purity
NADPH	Roche Diagnostic	10621706001	833.35	97%
UDPGA	Sigma	U6751	646.23	≥98
Alamethicin	Aladdin	A132913	1964.3078	99%

3. EXPERIMENTAL PROCEDURES

3.1 Stock solutions preparation:

1.90 mg of ALC-0159 was weighed and dissolved in 76 µL of DMSO to obtain a 10 mM stock solution. 2.547 mg of testosterone was weighed and dissolved in 551.93µL of DMSO to obtain a 16 mM solution. A 10 mM testosterone stock solution was then prepared by adding 60 µL DMSO to 100 µL of the 16 mM testosterone solution. 3.97 mg of 7-hydroxycoumarin was weighed and dissolved in 1224.25 µL of DMSO to obtain a 20 mM solution. A 10 mM 7-hydroxycoumarin stock solution was then prepared by adding 100 µL DMSO to 100 µL of the 20 mM 7-hydroxycoumarin solution. 5 mg of alamethicin was weighed and dissolved in 495 µL of 100 mM potassium phosphate buffer to obtain a 10 mg/mL alamethicin solution.

3.2 0.5 mM spiking solutions preparation:

Spiking Solution of Test Article or Positive Control			
Conc. of Stock Solution (mM)	Volume of Stock Solution (µL)	Volume of MeOH (µL)	Final Concentration (mM)
10	10	190	0.5

3.3 Preparation of 1.5× liver S9 suspensions (with alamethicin) containing test article or positive control:

1.5× Liver S9 Suspension (with Alamethicin) Containing Test Article or Positive Control						
Livers S9		0.5 mM Spiking Solution (µL)	10 mg/ml Alamethicin Solution	100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$ (pH 7.4) (µL)	Final Concentration	
Conc. of Stock Solution (mg/mL)	Volume of Stock Solution (µL)				Liver S9 Protein (mg/mL)	Compound (µM)
20	37.5	1.5	1.9	459.1	1.5	1.5

3.4 1.5× liver S9 suspensions (with alamethicin) containing test article or positive control were kept on ice for 15 minutes.

3.5 28.51 mg of NADPH was weighed and dissolved in 2.851 mL of 100 mM potassium phosphate buffer to obtain a 12 mM NADPH working solution. 25 mg of UDPGA was weighed and dissolved in 6.448 mL of 100 mM potassium phosphate buffer to obtain a 6 mM UDPGA working solution. 2.8 mL of 12 mM NADPH working solution was added into 2.8 mL of 6 mM UDPGA working solution to obtain a 6 mM NADPH and 3 mM UDPGA mixed working solution. This mixed working solution was then pre-warmed at 37ºC.

3.6 30µL of liver S9 suspension (with alamethicin) containing 1.5 µM test article or positive control was added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

3.7 96-well incubation plates were pre-warmed at 37ºC for 5 min.

3.8 For 0 min samples: 450 µL of ethanol containing internal standard (IS solution) was added before 15 µL of pre-warmed mixed working solution (6 mM NADPH and 3 mM UDPGA) was added.

3.9 For other samples (15, 30, 60, 90, and 120 min): 15 µL of pre-warmed mixed working solution (6 mM NADPH and 3 mM UDPGA) was added to initiate the reaction.

Volume (µL)			Final Concentration in Incubation Mixture		
1.5× Liver S9 Suspension Containing Test Article or Positive Control	3×NADPH Working Solution	Total	Liver S9 Protein (mg/mL)	Test Article or Positive Control (µM)	NADPH (mM)
30	15	45	0.5	1	2

The samples were incubated at 37ºC and 450 µL of IS solution was added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

3.10 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuge at 6,000 rpm for 15 min.

3.11 Then 200 µL of the supernatant from each well was transferred into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1 Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column: Agilent Zorbax SB-CN 3.5 µm (100 mm*2.1 mm)

Gradient for ALC-0159:

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

Solvent A: 0.1% formic acid in water

Solvent B: 0.1% formic acid in acetonitrile

Flow rate：600 µL/min

Column temperature：40ºC

Autosampler temperature: 4ºC

MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time (min)
ALC-0159	1164.00	494.70	45	71	~1.33
Tolbutamide (IS)	271.10	172.00	70	18	~1.03

4.3 Detection of ALC-0159

Representative chromatograms of ALC-0159 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0159 or positive control, testosterone and 7-hydroxycoumarin) was calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life were calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope
Half-life ($t_{1/2}$) = 0.693/k

6. RESULTS

A summary of the % remaining parent compound and half-life of ALC-0159 obtained from a 2-hour incubation with liver S9 from CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human is presented in Table 1. The stability of ALC-0159 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The liver S9 used in this study were tested for activity using metabolism control substrates under incubation conditions identical to those used for ALC-0159. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compounds (testosterone and 7-hydroxycoumarin) during the 2-hour incubation period, hence the test systems were considered to have yielded valid results. A summary of the % remaining parent compound and half-life of testosterone and 7-hydroxycoumarin is provided in Table 1. The stability of testosterone and 7-hydroxycoumarin over time in each matrix is shown in Figure 2 and Figure 3, respectively. Raw data for controls is presented in Appendix 3 (testosterone) and Appendix 4 (7-hydroxycoumarin).

7. CONCLUSIONS

This study evaluated the *in vitro* metabolic stability of ALC-0159 in liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 2-hour incubation with liver S9 from all these species.

Table 1. Summary of Liver S9 Stability of ALC-0159 , Testosterone and 7-Hydroxycoumarin

Compounds	Species		Percent Remaining (%)						$T_{1/2}$ (minute)
			0 min	15 min	30 min	60 min	90 min	120 min	
ALC-0159	CD-1/ICR Mouse	Mean	100.00	98.93	91.10	102.85	90.75	106.76	**>120**
		RSD of Area Ratio	0.02	0.03	0.03	0.00	0.04	0.03	
	Sprague Dawley Rat	Mean	100.00	84.38	90.87	97.97	93.51	92.70	**>120**
		RSD of Area Ratio	0.12	0.02	0.08	0.06	0.03	0.03	
	Cynomolgus Monkey	Mean	100.00	91.30	97.96	105.56	108.33	105.74	**>120**
		RSD of Area Ratio	0.02	0.08	0.01	0.11	0.05	0.13	
	Human	Mean	100.00	106.73	107.60	104.97	109.36	119.59	**>120**
		RSD of Area Ratio	0.05	0.00	0.01	0.00	0.01	0.03	
Testosterone	CD-1/ICR Mouse	Mean	100.00	59.38	35.71	6.89	BQL	BQL	**15.5**
		RSD of Area Ratio	0.05	0.11	0.01	0.16	N/A	N/A	
	Sprague Dawley Rat	Mean	100.00	BQL	BQL	BQL	BQL	BQL	**N/A**
		RSD of Area Ratio	0.05	N/A	N/A	N/A	N/A	N/A	
	Cynomolgus Monkey	Mean	100.00	81.12	68.58	45.76	27.19	16.74	**46.6**
		RSD of Area Ratio	0.11	0.11	0.02	0.06	0.02	0.08	
	Human	Mean	100.00	63.69	17.40	BQL	BQL	BQL	**11.9**
		RSD of Area Ratio	0.00	0.02	0.09	N/A	N/A	N/A	
7-Hydroxycoumarin	CD-1/ICR Mouse	Mean	100.00	40.06	16.68	3.57	1.89*	1.54*	**12.5**
		RSD of Area Ratio	0.00	0.11	0.02	0.17	0.18	0.20	
	Sprague Dawley Rat	Mean	100.00	71.60	48.00	26.17*	13.71*	11.92*	**28.3**
		RSD of Area Ratio	0.08	0.04	0.07	0.06	0.00	0.04	
	Cynomolgus Monkey	Mean	100.00	80.70	64.55	38.33	23.76	16.34	**44.8**
		RSD of Area Ratio	0.04	0.04	0.05	0.00	0.01	0.04	
	Human	Mean	100.00	69.01	43.17	19.16	8.29	3.68	**25.0**
		RSD of Area Ratio	0.02	0.00	0.04	0.03	0.03	0.12	

* The compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked with * were in the slow disappearance phase and were excluded from half-life calculation. BQL = Below quantification limit; N/A = not applicable

Figure 1. Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver S9

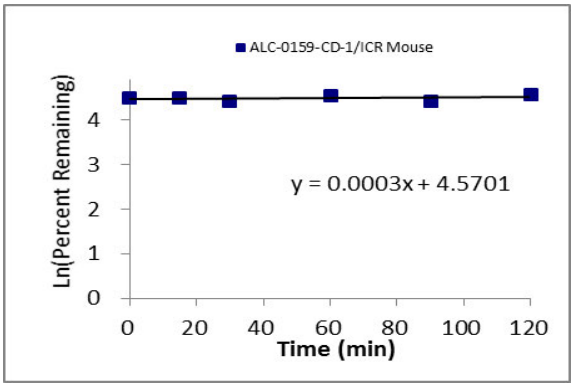
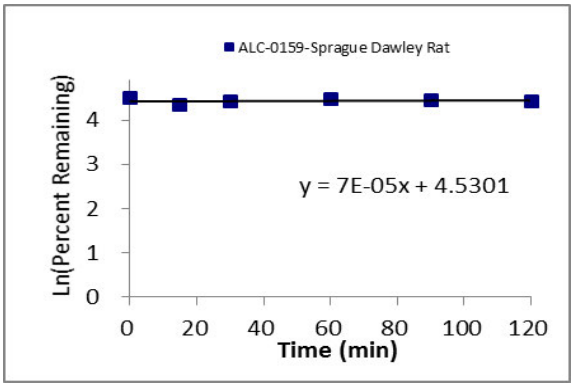
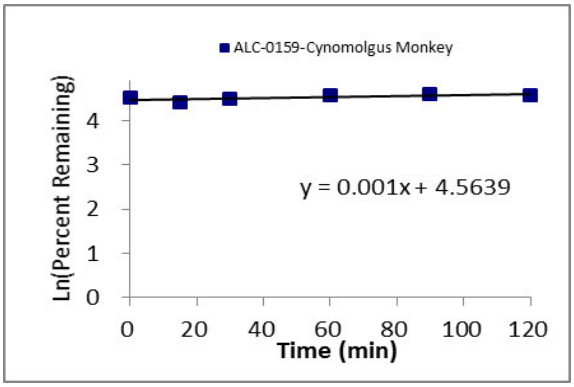
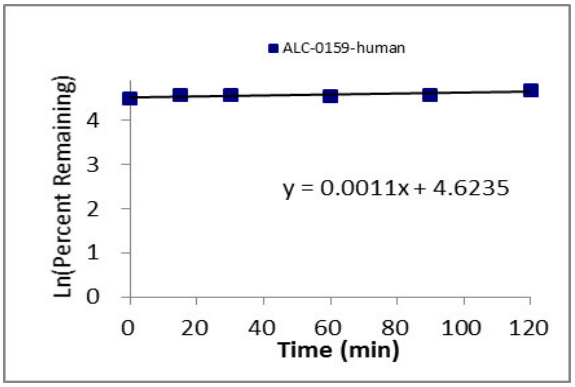
CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

Figure 2. Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9

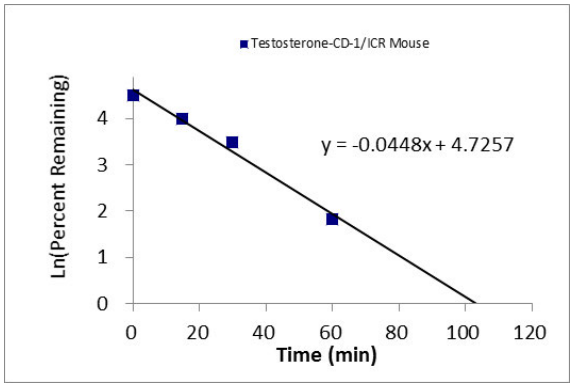
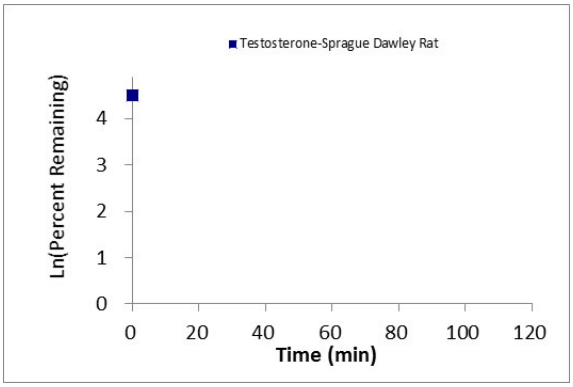
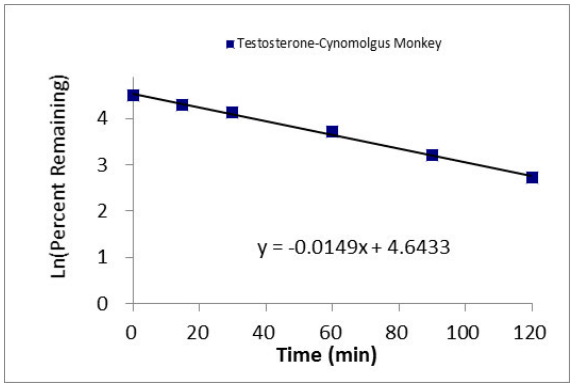
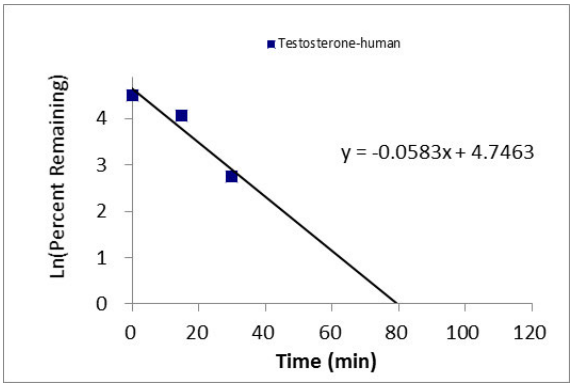
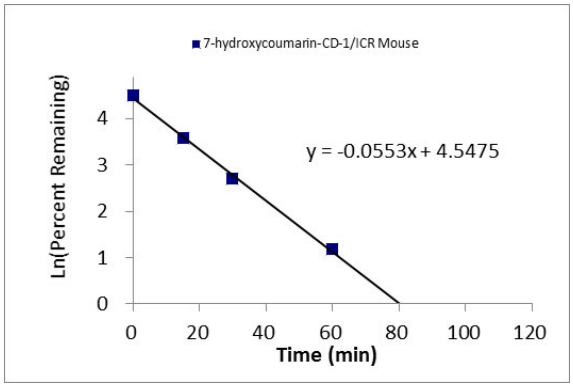
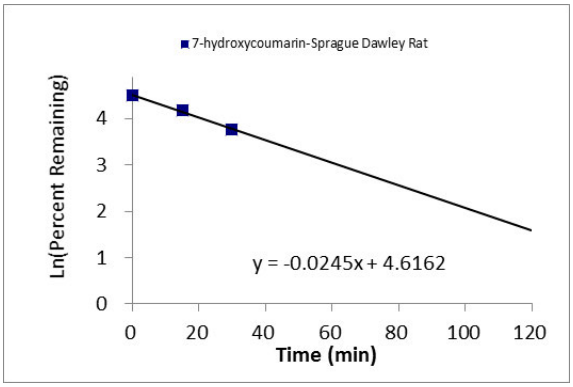
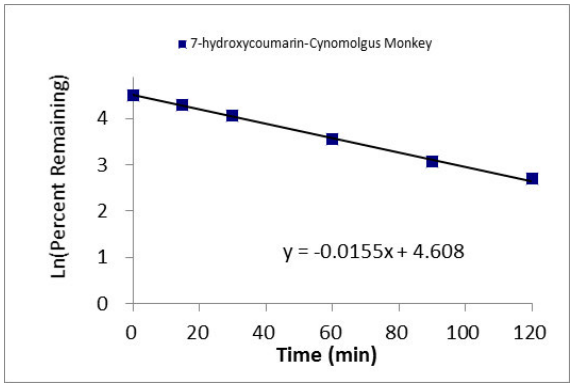
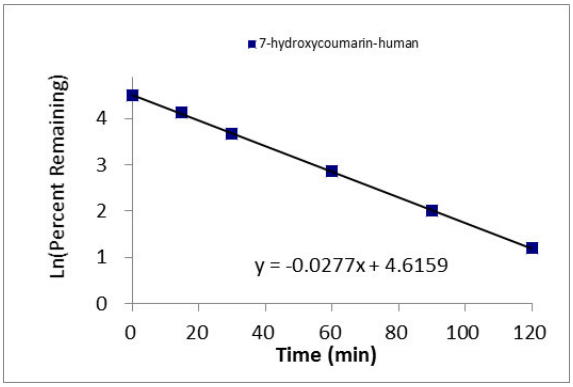
CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

Figure 3. Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9

CD-1/ICR Mouse	Sprague Dawley Rat
	
Cynomolgus Monkey	**Human**
	

8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Liver S9

Appendix 2 – Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Appendix 3 – Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

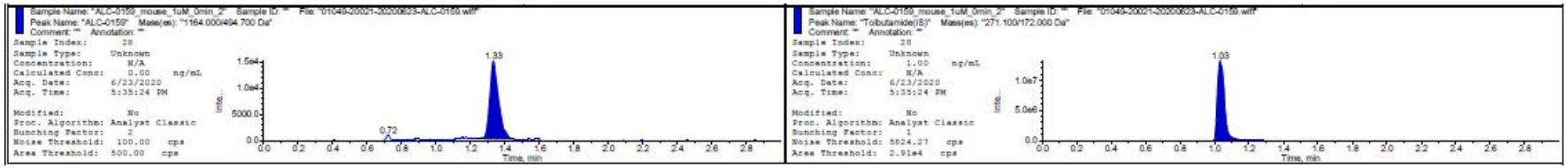
Appendix 4 – Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Appendix 5 – 01049-20021-S9 stability_protocol

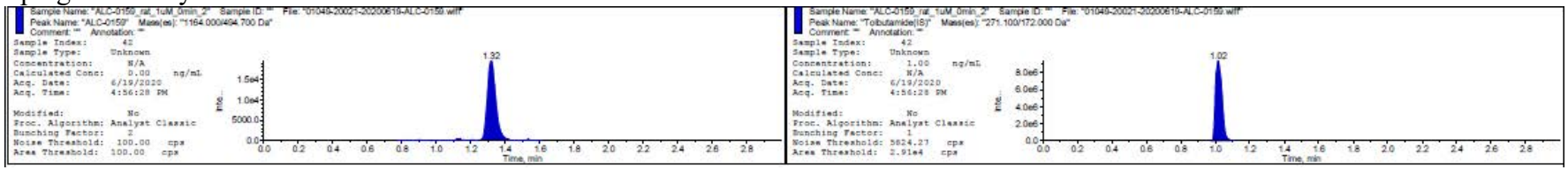
APPENDIX 1

Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Liver S9

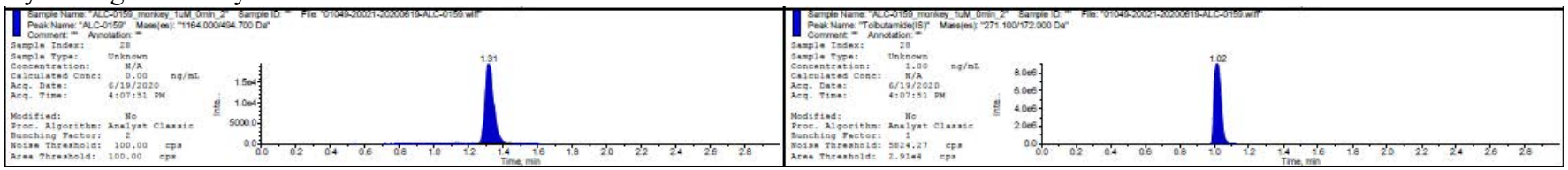
CD 1/ICR mouse



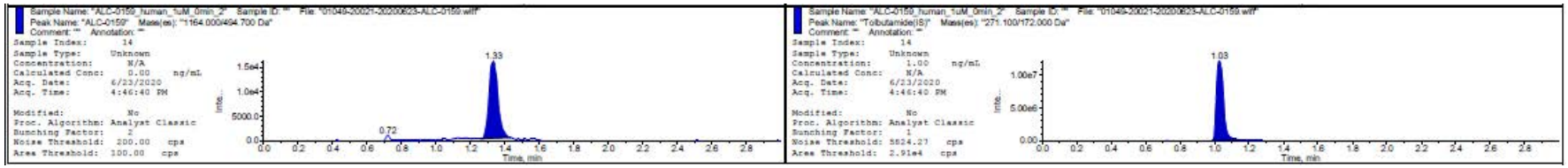
Sprague Dawley rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0159 in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0159	CD-1/ICR Mouse	0	5.08E+04	5.05E+04	3.57E+07	3.62E+07	0.001	0.001
		15	5.09E+04	4.82E+04	3.58E+07	3.55E+07	0.001	0.001
		30	4.48E+04	4.74E+04	3.58E+07	3.61E+07	0.001	0.001
		60	5.11E+04	5.11E+04	3.52E+07	3.56E+07	0.001	0.001
		90	4.47E+04	4.65E+04	3.59E+07	3.56E+07	0.001	0.001
		120	5.21E+04	5.42E+04	3.55E+07	3.55E+07	0.001	0.002
ALC-0159	Sprague Dawley Rat	0	5.46E+04	6.41E+04	2.42E+07	2.40E+07	0.002	0.003
		15	5.31E+04	5.16E+04	2.51E+07	2.51E+07	0.002	0.002
		30	5.82E+04	5.24E+04	2.46E+07	2.47E+07	0.002	0.002
		60	6.09E+04	5.58E+04	2.43E+07	2.40E+07	0.003	0.002
		90	5.90E+04	5.65E+04	2.49E+07	2.51E+07	0.002	0.002
		120	5.66E+04	5.43E+04	2.42E+07	2.44E+07	0.002	0.002
ALC-0159	Cynomolgus Monkey	0	6.60E+04	6.44E+04	2.41E+07	2.42E+07	0.003	0.003
		15	5.61E+04	6.26E+04	2.42E+07	2.40E+07	0.002	0.003
		30	6.35E+04	6.29E+04	2.39E+07	2.39E+07	0.003	0.003
		60	6.38E+04	7.33E+04	2.44E+07	2.38E+07	0.003	0.003
		90	7.46E+04	6.96E+04	2.47E+07	2.46E+07	0.003	0.003
		120	6.16E+04	7.28E+04	2.37E+07	2.34E+07	0.003	0.003
ALC-0159	Human	0	5.93E+04	5.62E+04	3.36E+07	3.40E+07	0.002	0.002
		15	6.18E+04	6.07E+04	3.37E+07	3.34E+07	0.002	0.002
		30	6.23E+04	6.19E+04	3.37E+07	3.38E+07	0.002	0.002
		60	6.08E+04	6.08E+04	3.40E+07	3.37E+07	0.002	0.002
		90	6.34E+04	6.28E+04	3.37E+07	3.38E+07	0.002	0.002
		120	7.07E+04	6.69E+04	3.38E+07	3.35E+07	0.002	0.002

APPENDIX 3

Stability of Testosterone in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Testosterone	CD-1/ICR Mouse	0	1.74E+04	1.71E+04	5.35E+05	5.68E+05	0.032	0.030
		15	1.06E+04	1.16E+04	6.22E+05	5.77E+05	0.017	0.020
		30	6.44E+03	6.29E+03	5.72E+05	5.69E+05	0.011	0.011
		60	1.10E+03	1.37E+03	5.79E+05	5.69E+05	0.002	0.002
		90	LOD	LOD	5.84E+05	5.74E+05	LOD	LOD
		120	LOD	LOD	5.70E+05	5.75E+05	LOD	LOD
Testosterone	Sprague Dawley Rat	0	1.52E+04	1.62E+04	6.17E+05	6.14E+05	0.025	0.026
		15	LOD	LOD	6.05E+05	5.74E+05	LOD	LOD
		30	LOD	LOD	6.53E+05	5.96E+05	LOD	LOD
		60	LOD	LOD	5.75E+05	5.65E+05	LOD	LOD
		90	LOD	LOD	6.06E+05	5.80E+05	LOD	LOD
		120	LOD	LOD	5.98E+05	6.00E+05	LOD	LOD
Testosterone	Cynomolgus Monkey	0	1.68E+04	1.43E+04	5.88E+05	5.86E+05	0.029	0.024
		15	1.22E+04	1.32E+04	6.16E+05	5.72E+05	0.020	0.023
		30	1.08E+04	1.08E+04	6.04E+05	5.85E+05	0.018	0.018
		60	6.82E+03	7.12E+03	5.88E+05	5.64E+05	0.012	0.013
		90	4.29E+03	4.11E+03	5.87E+05	5.79E+05	0.007	0.007
		120	2.78E+03	2.38E+03	5.94E+05	5.69E+05	0.005	0.004
Testosterone	Human	0	1.66E+04	1.57E+04	5.95E+05	5.63E+05	0.028	0.028
		15	1.02E+04	1.05E+04	5.81E+05	5.81E+05	0.018	0.018
		30	3.05E+03	2.85E+03	5.88E+05	6.27E+05	0.005	0.005
		60	LOD	LOD	5.92E+05	5.75E+05	LOD	LOD
		90	LOD	LOD	6.07E+05	6.32E+05	LOD	LOD
		120	LOD	LOD	5.50E+05	5.76E+05	LOD	LOD

LOD = Limit of detection

APPENDIX 4

Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Liver S9 – Raw Data

Compounds	Species	Time (min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
7-Hydroxycoumarin	CD-1/ICR Mouse	0	2.69E+04	2.63E+04	5.42E+05	5.29E+05	0.050	0.050
		15	1.20E+04	1.01E+04	5.59E+05	5.52E+05	0.021	0.018
		30	4.62E+03	4.53E+03	5.49E+05	5.54E+05	0.008	0.008
		60	1.11E+03	8.20E+02	5.54E+05	5.27E+05	0.002	0.002
		90	5.99E+02	4.34E+02	5.62E+05	5.30E+05	0.001	0.001
		120	3.69E+02	4.87E+02	5.59E+05	5.58E+05	0.001	0.001
7-Hydroxycoumarin	Sprague Dawley Rat	0	2.60E+04	2.51E+04	5.20E+05	5.61E+05	0.050	0.045
		15	1.88E+04	1.82E+04	5.37E+05	5.52E+05	0.035	0.033
		30	1.29E+04	1.20E+04	5.39E+05	5.52E+05	0.024	0.022
		60	6.94E+03	6.43E+03	5.38E+05	5.40E+05	0.013	0.012
		90	3.57E+03	3.60E+03	5.49E+05	5.56E+05	0.007	0.006
		120	2.92E+03	3.11E+03	5.32E+05	5.34E+05	0.005	0.006
7-Hydroxycoumarin	Cynomolgus Monkey	0	2.46E+04	2.60E+04	5.32E+05	5.32E+05	0.046	0.049
		15	2.18E+04	2.06E+04	5.53E+05	5.50E+05	0.039	0.037
		30	1.65E+04	1.74E+04	5.57E+05	5.45E+05	0.030	0.032
		60	9.83E+03	9.77E+03	5.40E+05	5.35E+05	0.018	0.018
		90	6.29E+03	6.06E+03	5.52E+05	5.40E+05	0.011	0.011
		120	4.19E+03	4.03E+03	5.23E+05	5.34E+05	0.008	0.008
7-Hydroxycoumarin	Human	0	2.65E+04	2.65E+04	5.32E+05	5.16E+05	0.050	0.051
		15	1.88E+04	1.83E+04	5.37E+05	5.24E+05	0.035	0.035
		30	1.23E+04	1.15E+04	5.49E+05	5.42E+05	0.022	0.021
		60	5.42E+03	5.06E+03	5.48E+05	5.33E+05	0.010	0.009
		90	2.28E+03	2.37E+03	5.55E+05	5.52E+05	0.004	0.004
		120	9.57E+02	1.11E+03	5.60E+05	5.53E+05	0.002	0.002

APPENDIX 5

01049-20021-S9 stability_protocol

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong
Shanghai 201299, China

Study Number
01049-20021

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20021

1.2. Study Title

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Cynomolgus Monkey, and Human Liver S9 Fractions

1.3. Sponsor Representative

(b) (6)

Acuitas Therapeutics Inc.

6190 Agronomy Road, Suite 402

Vancouver BC V6T 1Z3

Canada

(b) (6)

1.4. Objective

To evaluate the *in vitro* metabolic stability of ALC-0159 in liver S9 from different species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

[redacted]

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

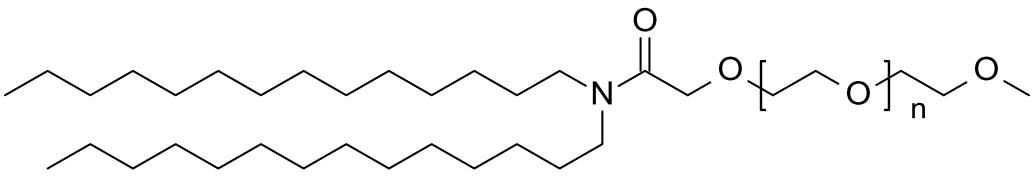
2. MATERIALS

2.1. Test Article

Name: ALC-0159

Molecular Formula: $C_{30}H_{60}NO\ (C_2H_4O)_n$ (n = 45~50)

MW (g/mol): ~2400-2600



2.2. Positive Control and Internal Standard

Testosterone and 7-hydroxycoumarin will be used as positive controls. Tolbutamide will be used as internal standard. The sources will be documented in experimental records and presented in the report.

2.3. Liver Microsomes and Cofactor

Liver S9 fractions of CD-1/ICR mouse, Sprague Dawley rat, cynomolgus monkey and human were purchased from qualified supplier and stored in a -70°C ultra low temperature freezer.

NADPH (reduced β-Nicotinamide adenine dinucleotide 2′-phosphate tetrasodium salt) were purchased from qualified supplier and stored at 2-8°C in a refrigerator.

UDPGA (uridine-diphosphate-glucuronic acid trisodium salt) and alamethicin were purchased from qualified suppliers and stored in a -20°C freezer.

The source and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solutions: Appropriate amount of test article or positive control is weighed and dissolved in DMSO to obtain a 10 mM stock solution.

(2) Preparation of 0.5 mM spiking solutions:

Spiking Solution of Test Article or Positive Control			
Conc. of stock solution	Volume of stock solution (μL)	Volume of MeOH (μL)	Final Concentration
10 mM	10	190	0.5 mM

(3) Preparation of 1.5× liver S9 suspensions with alamethicin containing test article or positive control:

1.5× Liver S9 Suspension with Alamethicin containing Test article or Positive control						
Livers S9		0.5 mM spiking solution (μL)	10 mg/ml Alamethicin	100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$ (pH 7.4) (μL)	Final Concentration	
Conc. of stock solution (mg/mL)	Volume of stock solution (μL)				Liver S9 protein (mg/mL)	Compound (μM)
20	37.5	1.5	1.9	459.1	1.5	1.5

(4) 1.5× liver S9 suspensions with alamethicin containing test article or positive control are kept on ice for 15 minutes.

(5) 3× master mix of cofactors (6 mM NADPH and 3 mM UDPGA in 100 mM potassium phosphate buffer containing 5 mM of $MgCl_2$, pH7.4) is prepared and then pre-warmed to 37ºC.

(6) 30 μL of liver S9 suspension with alamethicin containing 1.5 μM test article or positive control is added to 96-well plates in duplicate for each time point (0, 15, 30, 60, 90, and 120 min).

(7) 96-well incubation plates (from Step 6) are pre-warmed at 37 ºC for 5 min.

(8) For 0-min samples: 450 μL ethanol containing internal standard (IS solution) is added, followed by 15 μL pre-warmed 3× master mix of cofactors.

(9) For the 15, 30, 60, 90, and 120 min samples, 15 µL pre-warmed 3× master mix of cofactors is added to initiate reaction.

Volume of final incubation system (µL)			Final Concentration			
1.5× Liver S9 Suspension with Alamethicin containing Test article or Positive control	3× Master Mix of Cofactors	Total Volume	Liver S9 protein (mg/mL)	Compound (µM)	UDPGA (mM)	NADPH (mM)
30	15	45	1	1	1	2

The samples are incubated at 37 ºC and 450 µL IS solution (10ng/mL verapamil in ethanol) is added to stop the reaction at the corresponding time points (15, 30, 60, 90, and 120 min).

(10) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(11) The plates are sealed and stored at -20 ºC in a freezer until bioanalysis.

(12) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 µL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column：Agilent Zorbax SB-CN 3.5um (100mm*2.1mm)

Gradient Chromatography Parameters for ALC-0159:

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

Solvent A: 10mM ammonium formate, 0.1% Formic acid in water

Solvent B: 10mM ammonium formate, 0.1% Formic acid in acetonitrile

Flow rate：500 μL/min

Column temperature：40 ºC

Autosampler temperature: 4ºC

MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention
ALC-0159	1164.00	494.70	45	71	~1.30
Tolbutamide (IS)	271.10	172.00	70	18	~1.02

5. DATA ANALYSIS

The % remaining will be calculated by dividing the peak area ratio (test article peak area/ internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining will be plotted against time, and the slope of the regression line will be determined. Then elimination constant and half-life will be calculated as below.

Elimination rate constant (k) = - slope

Half-life ($t_{1/2}$) = 0.693/k

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

(b) (6)

June 17, 2020

Date

Sponsor Representative

Study Director Approval

(b) (6)

2020/06/17

Date

Study Director

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

Sponsor	Acuitas Therapeutics Inc. 6190 Agronomy Road, Suite 402 Vancouver BC V6T 1Z3 Canada
Testing Facility	Medicilon Preclinical Research (Shanghai) LLC 585 Chuanda Rd, Pudong Shanghai 201299 China
Study Monitor	(b) (6) Acuitas Therapeutics Inc. (b) (6)
Study Director	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Alternate Contact	(b) (6) Medicilon Preclinical Research (Shanghai) LLC (b) (6)
Study Identification	01049-20022
Experimental Start Date	2020-07-20
Experimental Completion Date	2020-07-22
Number of Pages in Report	32

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SUMMARY

This study evaluated the *in vitro* metabolic stability of ALC-0159 in hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 4-hour incubation with hepatocytes from all these species.

SIGNATURES

Compliance

This was a non-GLP study and was not conducted under full compliance with Good Laboratory Practice (GLP) regulations. Analyses were conducted according to an approved protocol and Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC. All data are documented in analysts' laboratory notebooks and electronic document management systems of Medicilon Preclinical Research (Shanghai) LLC. The content of this report has been reviewed against the raw data listings, summary tables and protocol for accuracy of the report.

Study Director Approval:

(b) (6)

Study Director

2020/08/10
Date

Sponsor Approval:

(b) (6)

Study Monitor

August 10, 2020
Date

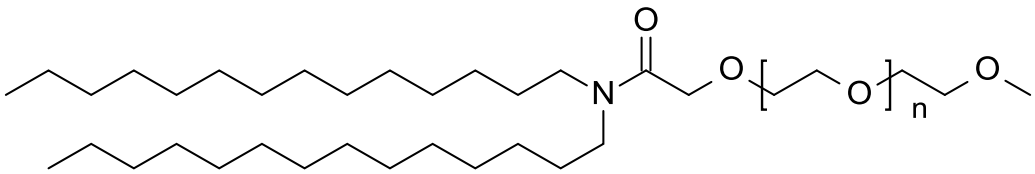
1. OBJECTIVE

To evaluate the *in vitro* metabolic stability of ALC-0159 in hepatocytes from different species.

2. MATERIALS

2.1 Test Article

Name: ALC-0159
Molecular Formula: C30H60NO (C2H4O) n (n = 45~50)
MW (g/mol): ~2400-2600



2.2 Positive Control

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Testosterone	Aladdin	58-22-0	T102169	K1505051	288.42
7-hydroxycoumarin	J&K Scientific	93-35-6	153384	L630I04	162.14

2.3 Internal Standard

Compound Name	Vendor	CAS No.	Cat. No.	Lot No.	Molecular Weight
Verapamil hydrochloride	TCI	152-11-4	V0118	RFMWJ-RL	491.06
Tolbutamide	Sigma-Aldrich	64-7-7	46968	BCBV8457	270.35

2.4 Hepatocytes

The following cryopreserved hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in liquid nitrogen until use.

Species	Manufacturer	Cat. No.	Lot No.	Assured Minimum Yield (cells per vial)
CD-1/ICR mouse (male)	XenoTech	MPCH1000	1810242	2.0×10^6
Sprague Dawley rat (male)	XenoTech	RPCH1000	1810189	5.0×10^6
Wistar Han rat	BioIVT	M00065	YMV	5.0×10^6
Cynomolgus monkey (male)	RILD Shanghai	HP-SXH-02M	CJJC	5.0×10^6
Human (mixed gender)	XenoTech	HPCH10	1810156	5.0×10^6

3. EXPERIMENTAL PROCEDURES

3.1 Stock solution:

4.24 mg of ALC-0159 was weighed and dissolved in 169.60 µL of DMSO to obtain a 10 mM stock solution. 3.31 mg of testosterone was weighed and dissolved in 1147.60 µL of DMSO to obtain a 10 mM stock solution. 2.81 mg of 7-hydroxycoumarin was weighed and dissolved in 882.70 µL of DMSO to obtain a 10 mM stock solution.

3.2 4 mM spiking solution:

Spiking Solution of Test Article or Positive Control				
Compound	Conc. of Stock Solution (mM)	Volume of Stock Solution (µL)	Volume of DMSO (µL)	Final Concentration (mM)
ALC-0159	10	20	30	4
Testosterone & 7-Hydroxycoumarin	10	20	10	4

3.3 2 µM dosing solution (2×):

Dosing Solution (2×) of Test Article or Positive Control			
Conc. of Spiking Solution (mM)	Volume of Spiking Solution (µL)	Volume of William's E Medium (µL)	Final Concentration (µM)
4	2	3998	2

3.4 Preparation of hepatocyte suspension:

Cryopreserved hepatocytes were thawed in a 37°C water bath, transferred to hepatocyte thawing medium (William's E Medium with 30% percoll and 5% FBS), and then centrifuged at 100×g for 10 min at room temperature. The cell pellet was resuspended with William's E Medium, cell viability was determined by trypan blue exclusion analysis, and the density of

viable cells was calculated. The hepatocytes were diluted with incubation medium to an appropriate density (2×10^6 viable cells/mL) and then pre-warmed at 37 °C for 10 min.

3.5 40 µL of each hepatocyte suspension was added to 96-well plates in duplicate for each time point (0, 30, 60, 90, 120, 180, and 240 min).

3.6 For 0 min samples: 480 µL of internal standard solution (IS solution, 10 ng/mL verapamil in ethanol) was added, followed by 40 µL of pre-warmed 2× dosing solution. The final concentration of test article or positive control in the incubation mixture was 1 µM.

3.7 For the 30, 60, 90, 120, 180, and 240 min samples, 40 µL of pre-warmed 2× dosing solution was added to initiate the reaction. The final concentration of test article or positive control in the incubation mixture was 1 µM.

3.8 Samples were incubated at 37 ℃ . At 30, 60, 90, 120, 180, and 240 min time points, the reaction was stopped by adding 480 µL ethanol containing internal standard to all of the duplicate wells.

3.9 After quenching, the plates were shaken at 600 rpm for 10 min and then centrifuged at 6,000 rpm for 15 min.

3.10 The plates were sealed and stored at -20 °C until bioanalysis.

3.11 Plates were thawed at room temperature, centrifuged at 6,000 rpm for 15 min, and 200 µL of the supernatants were transferred from each well into a 96-well sample plate for LC-MS/MS.

4. BIOANALYSIS

4.1 Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2 LC/MS/MS Conditions

Column: Agilent Zorbax SB-CN 3.5 µm (100 mm*2.1 mm)

Gradient for ALC-0159:

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in acetonitrile
Flow rate：600 μL/min
Column temperature：40 ºC
Autosampler temperature: 4ºC
MS Conditions: MRM detection

Compound	Q1(m/z)	Q3(m/z)	DP	CE	Retention Time
ALC-0159	1164.00	494.70	45	71	~1.33
tolbutamide (IS)	271.10	172.00	70	18	~1.03

4.3 Detection of ALC-0159

Representative chromatograms of ALC-0159 in each matrix are shown in Appendix 1.

5. DATA ANALYSIS

The % remaining parent compound (ALC-0159 or positive control, testosterone and 7-hydroxycoumarin) was calculated by dividing the peak area ratio (test article peak area/internal standard peak area) by the time zero peak area ratio. The natural logarithm of % remaining parent compound was plotted against time, and the slope of the regression line was determined. The elimination constant and half-life was calculated, when possible, as indicated below.

Elimination rate constant (k) = - slope
Half-life (T1/2) (minutes) = 0.693/k
Intrinsic clearance, predicted from the *in vitro* hepatocyte stability study, was calculated as shown below:

CL'int (mL/min/kg) = k × V (1 mL incubation/10^6 cells) × Scaling Factor (10^6 cells/kg),
Scaling Factor (10^6 cells/kg) = Hepatocellularity (10^6 cells/g liver) × Normalized Liver Weight (g liver/kg body weight)

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Hepatocytes

Species	Hepatocellularity (10^6 cells/g liver)	Liver Weight (g/kg BW)	Scaling Factor (10^6 cells/kg)
Mouse	135	87.5	11812.5
Rat	117	40	4680
Monkey	120	32	3840
Human	99	25.7	2544.3

6. RESULTS

A summary of the % remaining parent compound, CL'_{int} and half-life of ALC-0159 obtained from a 4-hour incubation with hepatocytes from CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human is presented in Table 2. The stability of ALC-0159 over time in each matrix is shown in Figure 1. Raw data is presented in Appendix 2.

The hepatocytes used in this study were tested for activity using metabolism control substrates under incubation conditions identical to those used for ALC-0159. The enzymes were found to exhibit satisfactory activity as determined by significant consumption of the positive control compounds (testosterone and 7-hydroxycoumarin) during the 4-hour incubation period, hence the test systems were considered to have yielded valid results. A summary of the % remaining parent compound, CL'_{int} and half-life of testosterone and 7-hydroxycoumarin is provided in Table 2. The stability of testosterone and 7-hydroxycoumarin over time in each matrix is shown in Figure 2 and Figure 3, respectively. Raw data for controls is presented in Appendix 3 (testosterone) and Appendix 4 (7-hydroxycoumarin).

7. CONCLUSIONS

This study evaluated the *in vitro* metabolic stability of ALC-0159 in hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human. ALC-0159 was stable after an approximately 4-hour incubation with hepatocytes from all these species.

Table 2. Summary of Hepatocyte Stability of ALC-0159, Testosterone and 7-Hydroxycoumarin

Test Article	Species		Percent Remaining (%)							$T_{1/2}$ (minute)	CL'int (mL/min/kg)
			0 min	30 min	60 min	90 min	120 min	180 min	240 min		
ALC-0159	CD-1/ICR mouse	Mean	100.00	100.85	94.92	94.28	87.08	94.92	102.75	**>240**	**<34.1**
		RSD of Area Ratio	0.60	4.16	1.89	0.95	3.10	0.63	3.21		
	Sprague Dawley rat	Mean	100.00	93.37	91.81	90.25	89.47	93.96	94.93	**>240**	**<13.5**
		RSD of Area Ratio	7.44	1.48	5.70	3.36	2.16	4.11	2.61		
	Wistar Han rat	Mean	100.00	113.04	105.07	112.80	104.11	102.90	98.79	**>240**	**<13.5**
		RSD of Area Ratio	3.42	2.42	4.23	3.94	5.58	0.00	3.11		
	Cynomolgus monkey	Mean	100.00	90.23	92.93	94.59	97.51	89.81	92.93	**>240**	**<11.3**
		RSD of Area Ratio	3.82	8.47	7.28	7.77	2.11	3.93	3.48		
	Human	Mean	100.00	106.34	101.58	92.67	96.04	93.66	102.57	**>240**	**<7.35**
		RSD of Area Ratio	1.96	0.79	1.93	5.44	0.87	3.89	6.55		
Testosterone	CD-1/ICR mouse	Mean	100.00	16.60	BQL	BQL	BQL	BQL	BQL	**11.6**	**707**
		RSD of Area Ratio	5.81	11.78	N/A	N/A	N/A	N/A	N/A		
	Sprague Dawley rat	Mean	100.00	7.23	BQL	BQL	BQL	BQL	BQL	**7.92**	**410**
		RSD of Area Ratio	3.17	N/A	N/A	N/A	N/A	N/A	N/A		
	Wistar Han rat	Mean	100.00	BQL	BQL	BQL	BQL	BQL	BQL	**N/A**	**N/A**
		RSD of Area Ratio	8.03	N/A	N/A	N/A	N/A	N/A	N/A		
	Cynomolgus monkey	Mean	100.00	10.07	BQL	BQL	BQL	BQL	BQL	**9.06**	**298**
		RSD of Area Ratio	2.81	41.26	N/A	N/A	N/A	N/A	N/A		
	Human	Mean	100.00	15.92	BQL	BQL	BQL	BQL	BQL	**11.3**	**156**
		RSD of Area Ratio	4.34	7.16	N/A	N/A	N/A	N/A	N/A		

7-Hydroxycoumarin	CD-1/ICR mouse	Mean	100.00	35.05	3.20	BQL	BQL	BQL	BQL	**12.1**	**677**
		RSD of Area Ratio	1.22	15.06	8.46	N/A	N/A	N/A	N/A		
	Sprague Dawley rat	Mean	100.00	20.97	BQL	BQL	BQL	BQL	BQL	**13.3**	**244**
		RSD of Area Ratio	2.99	10.49	N/A	N/A	N/A	N/A	N/A		
	Wistar Han rat	Mean	100.00	19.11	BQL	BQL	BQL	BQL	BQL	**12.6**	**258**
		RSD of Area Ratio	1.97	16.89	N/A	N/A	N/A	N/A	N/A		
	Cynomolgus monkey	Mean	100.00	17.03	BQL	BQL	BQL	BQL	BQL	**11.7**	**230**
		RSD of Area Ratio	0.85	2.27	N/A	N/A	N/A	N/A	N/A		
	Human	Mean	100.00	40.70	18.53	3.36	BQL	BQL	BQL	**24.7**	**71.5**
		RSD of Area Ratio	1.52	1.67	8.47	0.73	N/A	N/A	N/A		

* Compound showed biphasic metabolic kinetics, i.e., an initial fast disappearance phase was followed by a slow disappearance phase. The data points marked in * were in the slow disappearance phase and were excluded from half-life calculation.

BQL = Below quantification limit; N/A = not applicable

Figure 1. Stability of ALC-0159 in Mouse, Rat, Monkey and Human Hepatocytes

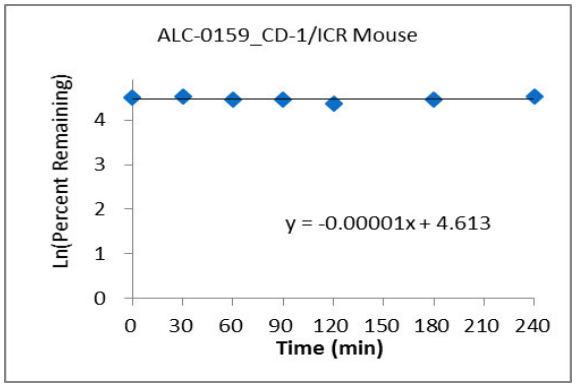
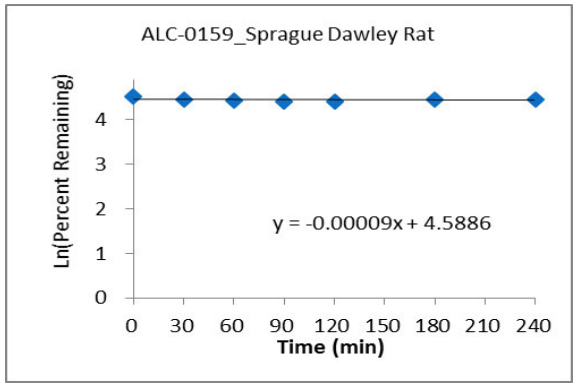
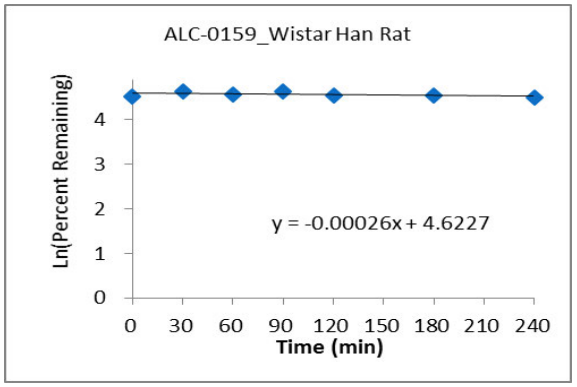
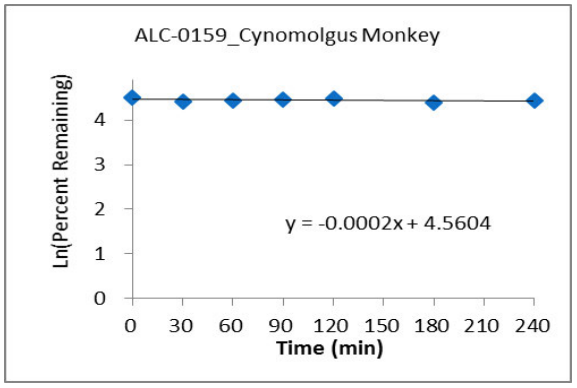
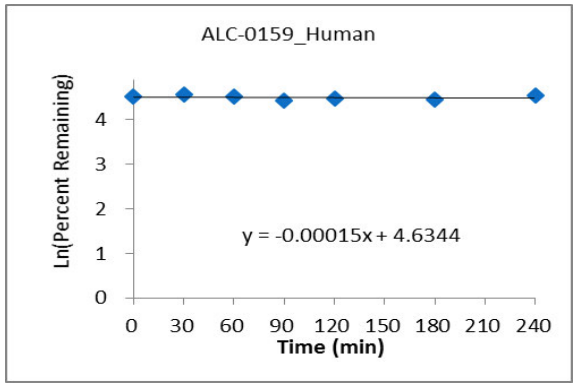
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 2. Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocytes

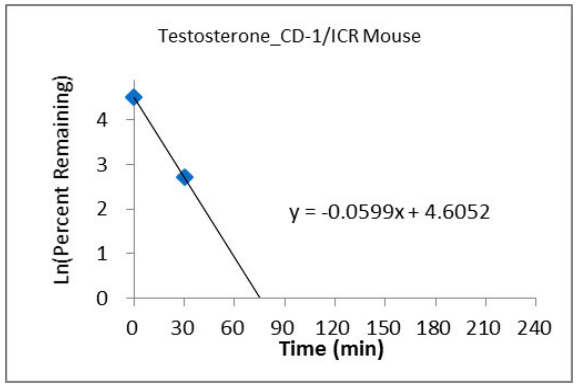
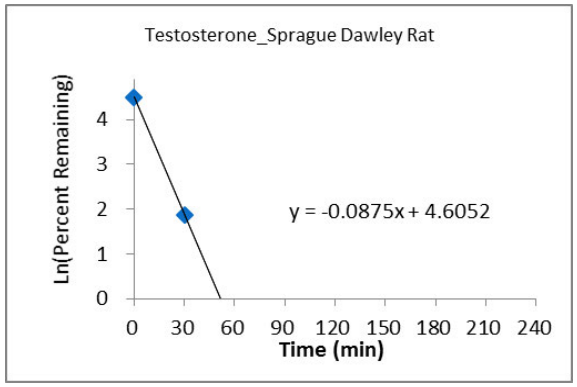
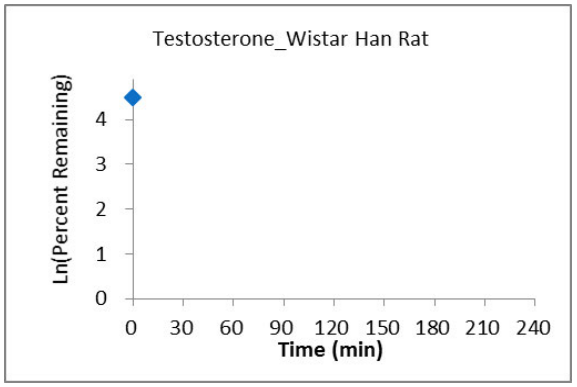
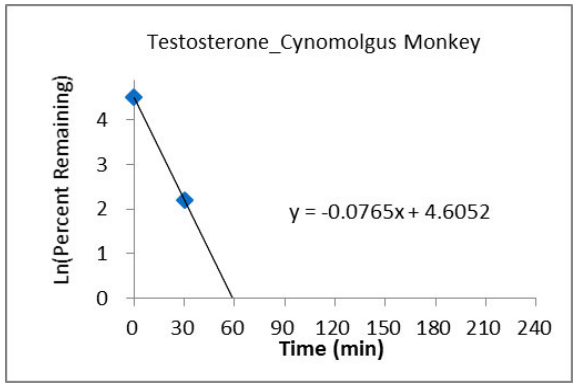
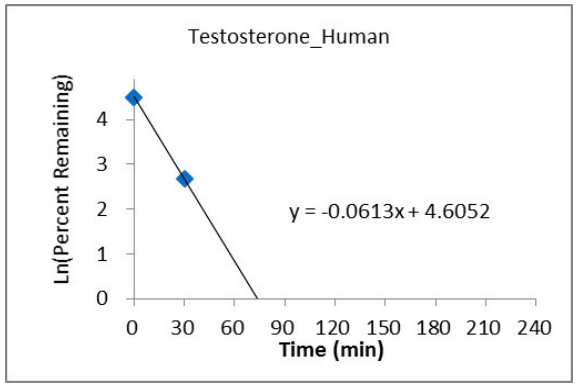
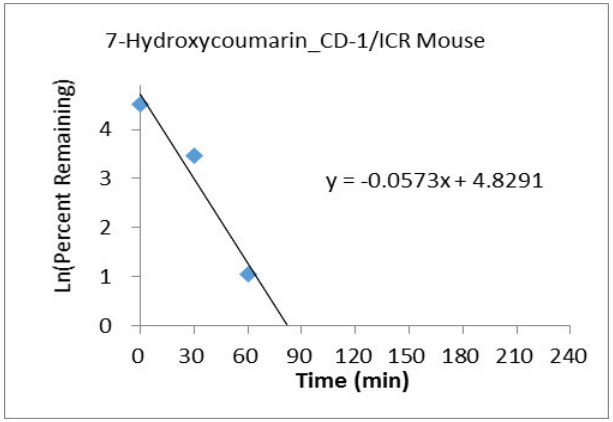
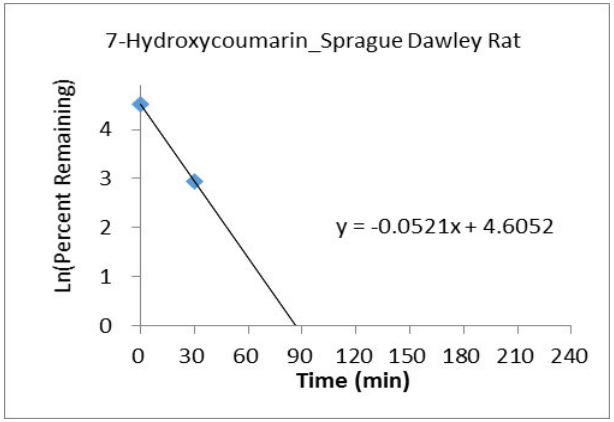
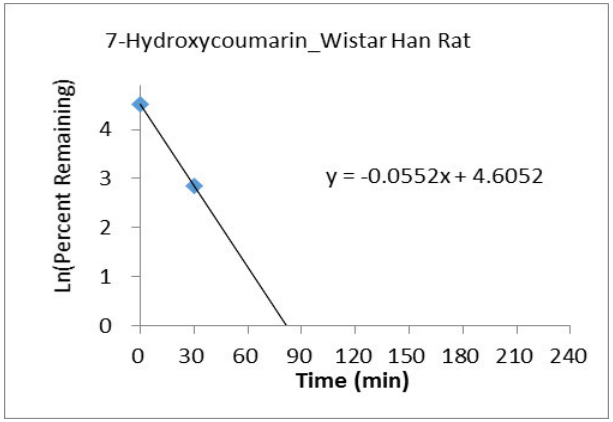
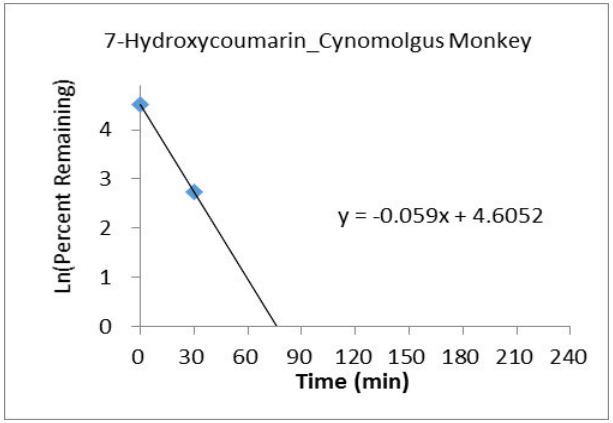
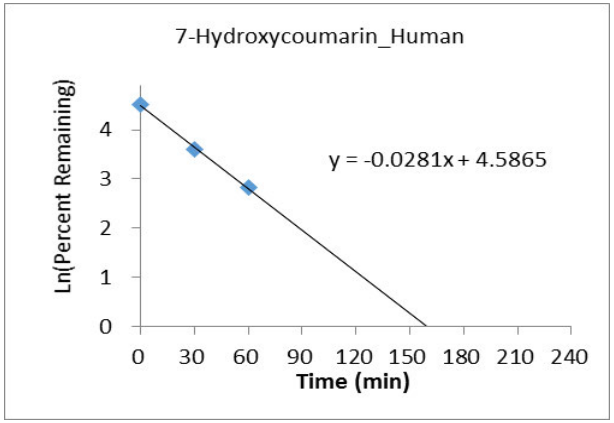
CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

Figure 3. Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocytes

CD-1/ICR Mouse	Sprague Dawley Rat	Wistar Han Rat
		
Cynomolgus Monkey	**Human**	
		

8. APPENDICES

Appendix 1 – Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Hepatocytes

Appendix 2 – Stability of ALC-0159 in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

Appendix 3 – Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

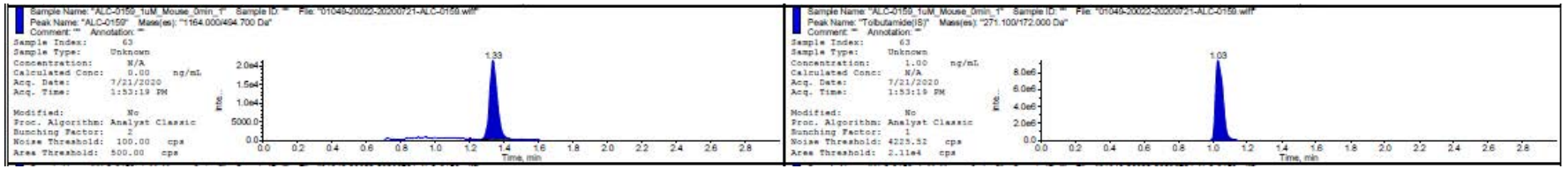
Appendix 4 – Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocytes – Raw Data

Appendix 5 – 01049-20022-ALC-0159-Hepatocytes Stability_Protocol

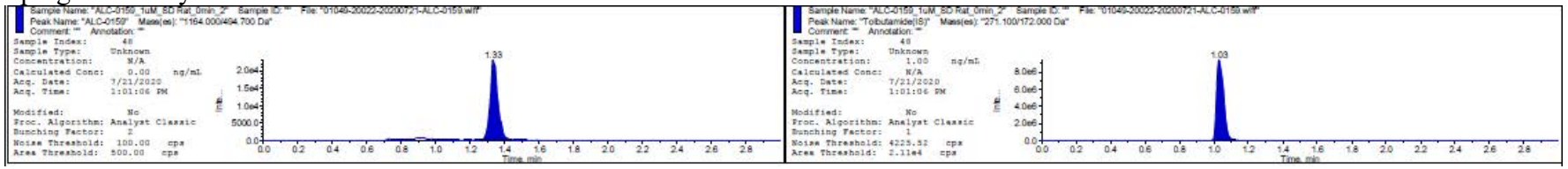
APPENDIX 1

Representative Chromatograms of ALC-0159 in Mouse, Rat, Monkey and Human Hepatocytes

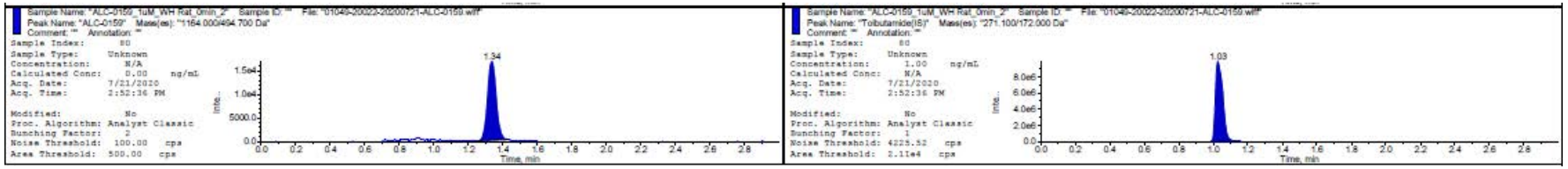
CD 1/ICR mouse



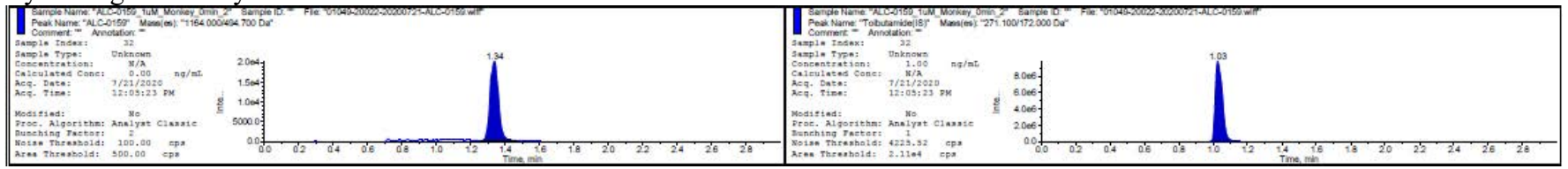
Sprague Dawley rat



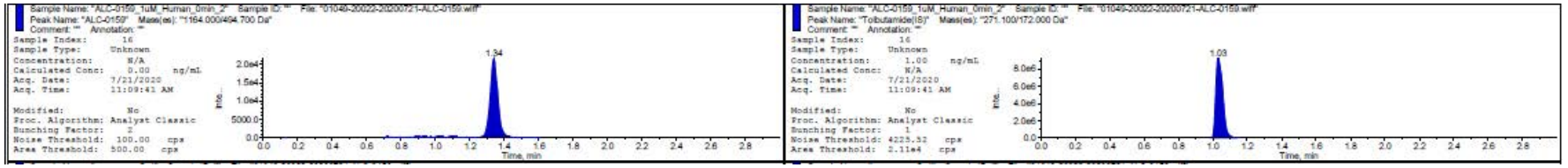
Wistar Han rat



Cynomolgus monkey



Human



APPENDIX 2

Stability of ALC-0159 in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
ALC-0159	CD-1/ICR mouse	240	6.41E+04	6.72E+04	2.70E+07	2.71E+07	0.002	0.002
		180	6.18E+04	6.20E+04	2.74E+07	2.78E+07	0.002	0.002
		120	5.40E+04	5.71E+04	2.69E+07	2.72E+07	0.002	0.002
		90	6.03E+04	5.93E+04	2.69E+07	2.69E+07	0.002	0.002
		60	6.06E+04	5.99E+04	2.67E+07	2.70E+07	0.002	0.002
		30	6.52E+04	6.18E+04	2.66E+07	2.68E+07	0.002	0.002
		0	6.30E+04	6.21E+04	2.66E+07	2.64E+07	0.002	0.002
ALC-0159	Sprague Dawley rat	240	6.44E+04	6.55E+04	2.69E+07	2.65E+07	0.002	0.002
		180	6.55E+04	6.23E+04	2.64E+07	2.66E+07	0.002	0.002
		120	5.92E+04	6.09E+04	2.61E+07	2.61E+07	0.002	0.002
		90	6.22E+04	5.93E+04	2.63E+07	2.63E+07	0.002	0.002
		60	6.40E+04	5.93E+04	2.61E+07	2.63E+07	0.002	0.002
		30	6.43E+04	6.22E+04	2.65E+07	2.62E+07	0.002	0.002
		0	6.39E+04	7.03E+04	2.63E+07	2.61E+07	0.002	0.003
ALC-0159	Wistar Han rat	240	5.74E+04	6.00E+04	2.86E+07	2.88E+07	0.002	0.002
		180	6.11E+04	6.01E+04	2.87E+07	2.82E+07	0.002	0.002
		120	5.87E+04	6.32E+04	2.83E+07	2.82E+07	0.002	0.002
		90	6.30E+04	6.59E+04	2.78E+07	2.75E+07	0.002	0.002
		60	6.12E+04	5.78E+04	2.73E+07	2.74E+07	0.002	0.002
		30	6.40E+04	6.59E+04	2.79E+07	2.77E+07	0.002	0.002
		0	5.89E+04	5.70E+04	2.78E+07	2.83E+07	0.002	0.002
ALC-0159	Cynomolgus monkey	240	6.16E+04	5.89E+04	2.69E+07	2.70E+07	0.002	0.002
		180	5.89E+04	5.65E+04	2.66E+07	2.69E+07	0.002	0.002
		120	6.19E+04	6.31E+04	2.68E+07	2.66E+07	0.002	0.002
		90	5.73E+04	6.50E+04	2.66E+07	2.71E+07	0.002	0.002
		60	5.73E+04	6.32E+04	2.70E+07	2.69E+07	0.002	0.002
		30	6.31E+04	5.59E+04	2.75E+07	2.74E+07	0.002	0.002
		0	6.32E+04	6.69E+04	2.70E+07	2.71E+07	0.002	0.002
ALC-0159	Human	240	6.22E+04	6.86E+04	2.52E+07	2.53E+07	0.002	0.003
		180	5.84E+04	6.26E+04	2.53E+07	2.58E+07	0.002	0.002
		120	6.12E+04	6.19E+04	2.51E+07	2.57E+07	0.002	0.002
		90	5.70E+04	6.08E+04	2.54E+07	2.50E+07	0.002	0.002
		60	6.56E+04	6.26E+04	2.52E+07	2.48E+07	0.003	0.003
		30	6.75E+04	7.09E+04	2.53E+07	2.63E+07	0.003	0.003
		0	6.53E+04	6.35E+04	2.55E+07	2.55E+07	0.003	0.002

APPENDIX 3

Stability of Testosterone in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
Testosterone	CD-1/ICR mouse	240	LOD	LOD	7.53E+05	7.48E+05	LOD	LOD
		180	LOD	LOD	7.77E+05	7.83E+05	LOD	LOD
		120	LOD	LOD	7.44E+05	7.99E+05	LOD	LOD
		90	LOD	LOD	7.60E+05	7.89E+05	LOD	LOD
		60	LOD	LOD	7.39E+05	7.46E+05	LOD	LOD
		30	5.29E+03	6.16E+03	7.70E+05	7.58E+05	0.007	0.008
		0	3.64E+04	3.41E+04	7.73E+05	7.88E+05	0.047	0.043
Testosterone	Sprague Dawley rat	240	LOD	LOD	8.19E+05	8.01E+05	LOD	LOD
		180	LOD	LOD	7.97E+05	7.54E+05	LOD	LOD
		120	LOD	LOD	7.48E+05	8.25E+05	LOD	LOD
		90	LOD	LOD	8.12E+05	7.45E+05	LOD	LOD
		60	LOD	LOD	7.59E+05	7.44E+05	LOD	LOD
		30	LOD	2.38E+03	8.25E+05	8.19E+05	LOD	0.003
		0	3.38E+04	3.38E+04	8.23E+05	8.59E+05	0.041	0.039
Testosterone	Wistar Han rat	240	LOD	LOD	7.72E+05	8.57E+05	LOD	LOD
		180	LOD	LOD	7.61E+05	7.44E+05	LOD	LOD
		120	LOD	LOD	7.87E+05	7.53E+05	LOD	LOD
		90	LOD	LOD	7.87E+05	7.71E+05	LOD	LOD
		60	LOD	LOD	7.29E+05	7.93E+05	LOD	LOD
		30	LOD	LOD	7.78E+05	7.87E+05	LOD	LOD
		0	3.34E+04	3.39E+04	8.20E+05	7.44E+05	0.041	0.046
Testosterone	Cynomolgus monkey	240	LOD	LOD	8.17E+05	8.22E+05	LOD	LOD
		180	LOD	LOD	8.26E+05	8.16E+05	LOD	LOD
		120	LOD	LOD	8.22E+05	8.12E+05	LOD	LOD
		90	LOD	LOD	8.44E+05	7.91E+05	LOD	LOD
		60	LOD	LOD	8.47E+05	7.85E+05	LOD	LOD
		30	4.32E+03	2.37E+03	8.24E+05	8.22E+05	0.005	0.003
		0	3.45E+04	3.26E+04	8.72E+05	7.93E+05	0.04	0.041
Testosterone	Human	240	LOD	LOD	8.02E+05	8.22E+05	LOD	LOD
		180	LOD	LOD	8.65E+05	8.75E+05	LOD	LOD
		120	LOD	LOD	8.29E+05	8.22E+05	LOD	LOD
		90	LOD	LOD	8.60E+05	8.16E+05	LOD	LOD
		60	LOD	LOD	8.21E+05	8.47E+05	LOD	LOD
		30	6.13E+03	5.10E+03	8.78E+05	8.09E+05	0.007	0.006
		0	3.25E+04	3.56E+04	8.02E+05	8.26E+05	0.04	0.043

LOD = limit of detection

APPENDIX 4

Stability of 7-Hydroxycoumarin in Mouse, Rat, Monkey and Human Hepatocyte – Raw Data

Compound	Species	Time(min)	Raw Data					
			Analyte Peak Area (counts)	Analyte Peak Area (counts)	IS Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Area Ratio
7-Hydroxycoumarin	CD-1/ICR mouse	240	LOD	LOD	6.12E+05	6.29E+05	LOD	LOD
		180	LOD	LOD	6.12E+05	6.09E+05	LOD	LOD
		120	LOD	LOD	6.11E+05	5.99E+05	LOD	LOD
		90	LOD	LOD	6.29E+05	6.06E+05	LOD	LOD
		60	1.33E+03	1.21E+03	6.10E+05	6.25E+05	0.002	0.002
		30	1.25E+04	1.57E+04	6.23E+05	6.31E+05	0.02	0.025
		0	3.97E+04	4.12E+04	6.25E+05	6.37E+05	0.064	0.065
7-Hydroxycoumarin	Sprague Dawley rat	240	LOD	LOD	6.30E+05	6.18E+05	LOD	LOD
		180	LOD	LOD	6.29E+05	6.25E+05	LOD	LOD
		120	LOD	LOD	6.36E+05	6.49E+05	LOD	LOD
		90	LOD	LOD	6.11E+05	6.30E+05	LOD	LOD
		60	LOD	LOD	6.19E+05	6.07E+05	LOD	LOD
		30	8.21E+03	9.55E+03	6.30E+05	6.32E+05	0.013	0.015
		0	3.98E+04	4.10E+04	6.06E+05	5.99E+05	0.066	0.068
7-Hydroxycoumarin	Wistar Han rat	240	LOD	LOD	6.23E+05	6.17E+05	LOD	LOD
		180	LOD	LOD	6.51E+05	6.11E+05	LOD	LOD
		120	LOD	LOD	6.05E+05	6.24E+05	LOD	LOD
		90	LOD	LOD	6.10E+05	6.15E+05	LOD	LOD
		60	LOD	LOD	6.36E+05	6.05E+05	LOD	LOD
		30	6.78E+03	8.59E+03	6.20E+05	6.18E+05	0.011	0.014
		0	4.01E+04	3.94E+04	6.09E+05	6.14E+05	0.066	0.064
7-Hydroxycoumarin	Cynomolgus monkey	240	LOD	LOD	5.82E+05	6.25E+05	LOD	LOD
		180	LOD	LOD	6.01E+05	6.18E+05	LOD	LOD
		120	LOD	LOD	6.38E+05	6.14E+05	LOD	LOD
		90	LOD	LOD	6.38E+05	6.07E+05	LOD	LOD
		60	LOD	LOD	6.28E+05	6.20E+05	LOD	LOD
		30	7.22E+03	6.96E+03	6.42E+05	6.39E+05	0.011	0.011
		0	4.21E+04	4.15E+04	6.44E+05	6.43E+05	0.065	0.065
7-Hydroxycoumarin	Human	240	LOD	LOD	6.04E+05	6.05E+05	LOD	LOD
		180	LOD	LOD	6.45E+05	6.24E+05	LOD	LOD
		120	LOD	LOD	6.28E+05	6.50E+05	LOD	LOD
		90	1.43E+03	1.40E+03	6.42E+05	6.21E+05	0.002	0.002
		60	7.22E+03	8.24E+03	6.20E+05	6.28E+05	0.012	0.013
		30	1.69E+04	1.68E+04	6.27E+05	6.10E+05	0.027	0.028
		0	4.06E+04	3.99E+04	6.01E+05	6.03E+05	0.068	0.066

LOD = limit of detection

APPENDIX 5

01049-20022-ALC-0159-Hepatocytes Stability_Protocol

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

Testing Facility
Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road
Pudong, Shanghai 201299
China

Study Number
01049-20022

Study Director
(b) (6)

Sponsor
Acuitas Therapeutics Inc.

CONTENTS

1. INTRODUCTION

1.1. Study Number

01049-20022

1.2. Study Title

In Vitro Metabolic Stability of ALC-0159 in CD-1/ICR Mouse, Sprague Dawley Rat, Wistar Han Rat, Cynomolgus Monkey, and Human Hepatocytes

1.3. Sponsor Representative

(b) (6)

Acuitas Therapeutics Inc.
6190 Agronomy Road, Suite 402
Vancouver BC V6T 1Z3
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(b) (6)

1.4. Objective

To evaluate the in vitro metabolic stability of ALC-0159 in Hepatocytes from different species and to determine intrinsic clearance in each species.

1.5. Compliance

This is a non-GLP study and will be conducted according to the Standard Operating Procedures (SOPs) of Medicilon Preclinical Research (Shanghai) LLC.

1.6. Testing Facility

Medicilon Preclinical Research (Shanghai) LLC
585 Chuanda Road, Pudong, Shanghai 210299, China

1.7. Personnel

1.7.1. Study Director

(b) (6)

1.7.2. Alternate Contact

(b) (6)

1.8. Study Schedule

Study Initiation Date:	Signature date by Study Director
Experiment Start Date:	To be included in the final report
Experiment Termination Date:	To be included in the final report
Draft Report Issue Date:	To be included in the final report

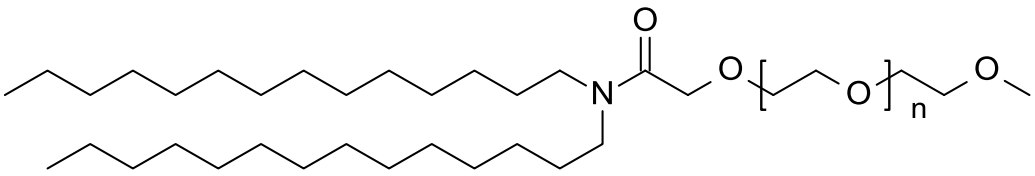
2. MATERIALS

2.1. Test Article

Name: ALC-0159

Molecular Formula: $C_{30}H_{60}NO\ (C_2H_4O)_n$ (n = 45~50)

MW (g/mol): ~2400-2600



2.2. Positive Control and Internal Standard

Testosterone and 7-hydroxycoumarin will be used as positive controls. Tolbutamide will be used as internal standard. The sources will be documented in the experimental records and presented in the report.

2.3. Hepatocytes

Cryopreserved hepatocytes of CD-1/ICR mouse, Sprague Dawley rat, Wistar Han rat, cynomolgus monkey, and human were purchased from qualified suppliers and stored in liquid nitrogen until use. The source(s) and lot numbers will be documented in the experimental records and presented in the final report.

3. EXPERIMENTAL PROCEDURES

(1) Preparation of stock solution: Appropriate amount of test article or positive control is weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain a 10 mM stock solution.

(2) Preparation of 4 mM spiking solution:

Spiking Solution of Test Article or Positive Control			
Conc. of Stock Solution (mM)	Volume of Stock Solution (μL)	Volume of DMSO (μL)	Final Concentration (mM)
10	20	30	4

(3) Preparation of 2 μM dosing solution(2×) of test article or positive control:

Dosing Solution (2×) of Test Article or Positive Control			
Conc. of Spiking Solution (mM)	Volume of Spiking Solution (μL)	Volume of William's E Medium (μL)	Final Concentration (μM)
4	2	3998	2

(4) Preparation of hepatocyte suspension: Thaw cryopreserved hepatocytes in a 37℃ water bath. Transfer the hepatocytes to hepatocyte thawing medium (William's E Medium with 30% percoll and 5% FBS) and centrifuge at 100×g for 10 min at room temperature. Resuspend the cell pellet with William's E Medium and determine cell viability by trypan blue exclusion analysis and calculate the viable cell density. Dilute the hepatocytes with incubation medium to an appropriate density (2×10^6 viable cells/mL) and pre-warm at 37 °C for 10 min.

(5) 40 μL of each hepatocyte suspension is added to 96-well plates in duplicate for each time point (0, 30, 60, 90, 120, 180, and 240 min).

(6) For 0 min samples: 480 μL of internal standard solution (IS solution, 10 ng/mL verapamil in ethanol) is added, followed by 40 μL of pre-warmed 2× dosing solution. The final concentration of test article or positive control in the incubation mixture is 1 μM.

(7) For the 30, 60, 90, 120, 180, and 240 min samples, 40 μL of pre-warmed 2× dosing solution is added to initiate reaction. The final concentration of test article or positive control in the incubation mixture is 1 μM.

(8) The samples are incubated at 37℃ . At 30, 60, 90, 120, 180, and 240 min time points, stop the reaction by adding 480 μL ethanol containing internal standard to all of the duplicate wells.

(9) After quenching, shake the plates at 600 rpm for 10 min and then centrifuge them at 6,000 rpm for 15 min.

(10) The plates are sealed and stored at -20℃ freezer until bioanalysis.

(11) Thaw the plates at room temperature, centrifuge them at 6,000 rpm for 15 min, then transfer 200 μL of the supernatant from each well into a 96-well sample plate for LC-MS/MS analysis.

4. BIOANALYSIS

4.1. Instruments

SHIMADZU：UPLC system

Sciex Triple Quad 6500+ with ESI ion source

4.2. LC/MS/MS Conditions

Column: Agilent Zorbax SB-CN 3.5um (100mm*2.1mm)

Gradient for ALC-0159

Time (min)	Solvent A (%)	Solvent B (%)
0.00	80	20
0.40	30	70
1.60	10	90
2.70	10	90
2.71	80	20
3.00	80	20

A: 0.1%Formic acid in water

B: 0.1%Formic acid in acetonitrile

Flow rate：600 μL/min

Column temperature：40℃

Autosampler temperature: 4ºC

Compound	Q1(m/z)	Q3(m/z)	Retention Time (min)
ALC-0159	1164.00	494.70	~1.30
Tolbutamide (IS)	271.10	172.00	~1.02

5. DATA ANALYSIS

The % remaining (parent compound) will be calculated by dividing the peak area ratio (compound peak area/ internal standard peak area) by the 0 min peak area ratio. The natural logarithm of % remaining is plotted against time and the slope of the fitted line will be determined as follows:

Elimination rate constant (k) = - slope

Half-life ($T_{1/2}$) (minutes) = 0.693/k

Intrinsic clearance predicted from the *in vitro* hepatocyte stability study will be calculated as shown below:

CL'_{int} (mL/min/kg) = k * V (1 mL incubation/10^6 cells) * Scaling Factor (10^6 cells/kg),

Scaling Factor (10^6 cells/kg) = Hepatocellularity (10^6 cells/g liver) * Normalized Liver Weight (g liver/kg body weight)

The scaling factors are listed in Table 1.

Table 1. Scaling Factors for Intrinsic Clearance Prediction in Mouse, Rat, Monkey, and Human Hepatocytes

Species	Hepatocellularity (10^6 cells/g liver)	Liver Weight (g/kg BW)	Scaling Factor (10^6 cells/kg)
Mouse	135	87.5	11812.5
Rat	117	40	4680
Monkey	120	32	3840
Human	99	25.7	2544.3

6. FINAL REPORT

After completion of the study, a draft report including the results, analysis and discussion will be sent to the Sponsor in Microsoft Word format.

One month after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report will be issued as a final report, signed by the Study Director, and submitted to the Sponsor in Adobe Acrobat PDF format, containing hyperlinks, as applicable. Any modifications or changes to the draft report requested one month after issuance of the draft will be performed at additional cost to the Sponsor.

7. SIGNATURES

(b) (6)

Sponsor Representative

July 15, 2020

Date

Study Director Approval

(b) (6)

Study Director

2020/07/15

Date

INVESTIGATION OF THE BIOTRANSFORMATION OF ALC-0159 AND ALC-0315 IN VITRO AND IN VIVO IN RATS

LIST OF ABBREVIATIONS

Abbreviation	Term
ALC-0159	Proprietary PEG-lipid included as an excipient in the LNP formulation used in the COVID-19 mRNA vaccine
ALC-0315	Proprietary amino-lipid included as an excipient in the LNP formulation used in the COVID-19 mRNA vaccine
COVID-19	Coronavirus disease 2019
DMSO	Dimethyl sulfoxide
LNP	Lipid-nanoparticles
MeCN	Acetonitrile
modRNA	Nucleoside-modified mRNA
mRNA	Messenger RNA
NAD+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP+	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
PAPS	3'-Phosphoadenosine-5'-phosphosulfate
PEG	Polyethylene glycol
S9	Supernatant obtained from liver homogenate by centrifuging at 9000g
UDPGA	Uridine diphosphate glucuronic acid
UHPLC	Ultra high-performance liquid chromatography

1. ABSTRACT

The metabolism of the novel excipients, ALC-0159 and ALC-0315, was examined *in vitro* using blood, liver S9 fractions and hepatocytes, all from mouse, rat, monkey and human. The *in vivo* metabolism was examined in rat plasma, urine, feces, and liver from a rat pharmacokinetics study where a luciferase-encoding mod RNA formulated in LNP with an identical lipid composition as PF-07302048 (COVID-19 mRNA Vaccine; BioNTech code number BNT162) was administered intravenously at a 1 mg/kg dose.

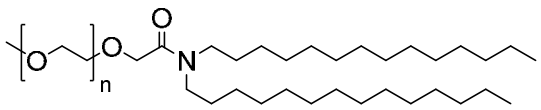
The primary route of metabolism identified for ALC-0159 involves amide bond hydrolysis yielding *N*,*N*-ditetradecylamine (*m/z* 410). This metabolite was identified in mouse and rat blood, as well as hepatocytes and liver S9 from mouse, rat, monkey and human. No metabolites of ALC-0159 were identified from *in vivo* samples.

Metabolism of ALC-0315 occurs via two sequential ester hydrolysis reactions, first yielding the monoester metabolite (*m/z* 528) followed by the doubly deesterified metabolite (*m/z* 290). The monoester metabolite was observed *in vitro* in rat blood, monkey S9 fraction, and *in vivo* in rat plasma and rat liver. The doubly deesterified metabolite was observed *in vitro* in mouse and rat blood; monkey liver S9 fraction; and *in vivo* in rat plasma, urine, feces and liver. Subsequent metabolism of the doubly deesterified metabolite resulted in a glucuronide metabolite (*m/z* 466) which was observed in urine only from the rat pharmacokinetics study. Additionally, 6-hexyldecanoic acid (*m/z* 255), the acid product of both ester hydrolysis reactions of ALC-0315, was identified *in vitro* in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma.

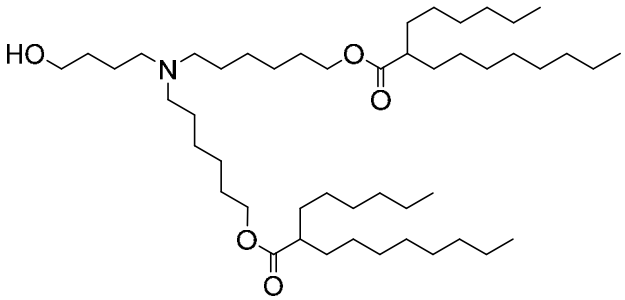
Based on nonquantitative ion current data for parent depletion and metabolite formation, metabolism for both ALC-0159 and ALC-0315 appears to occur relatively slowly across most species *in vitro* and *in vivo*. Overall, it can be concluded that both ALC-0159 and ALC-0315 are metabolized by hydrolytic metabolism of the amide or ester functionalities, respectively, and this hydrolytic metabolism is observed across the species evaluated.

2. OBJECTIVES

The objective of this study was to provide a preliminary qualitative assessment of the biotransformation of the novel excipients ALC-0159 and ALC-0315 in blood, liver S9 fractions and hepatocytes from mouse, rat, monkey and human as well as in plasma, urine, feces and liver samples from a rat pharmacokinetics study.



ALC-0159 (n = 40-51)
n = 45, major component



ALC-0315

3. MATERIALS AND METHODS

3.1. Materials

ALC-0159 (2-[(polyethylene glycol]-2000]-*N*,*N*-ditetradecylacetamide, Lot# GALC0159-10), ALC-0315 ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl) bis(2-hexyldecanoate), Lot# GALC0315-11), and Carboxy-MPEG2 (methoxypolyethylene glycol 2000 acetic acid, Lot# 792354-01-011) were obtained from Avanti Polar Lipids, Inc. NAD+, reduced NADH, reduced NADPH, NADP+, alamethicin, adipic acid, diethylene glycol, triethylene glycol, tetraethylene glycol, myristic acid, tetradecylamine, 6-hexyldecanoic acid, 4-aminobutyric acid, and 6-aminohexanoic acid were obtained from Millipore-Sigma (St. Louis, MO). *N*,*N*-Ditetradecylamine was obtained from Ambeed (Arlington Heights, IL). All other reagents were the highest grade commercially available.

Blood from mouse (female, CD-1), rat (male, Wistar Han), monkey (male, cynomolgus), and human (one male and one female) was obtained from in-house untreated animals and from human donors not taking any medications. Potassium EDTA (K_2EDTA) was used as the anticoagulant for all species. In all species except rat, the blood used for the *in vitro* assessments was a pool of 2 or more animals or donors. Mouse (male, CD-1, BioIVT, lot YKA), rat (male, Wistar Han, BioIVT, lot DTO), monkey (male, cynomolgus, BioIVT, lot DNB), and human (mixed gender, BioIVT, lot SPB) hepatocytes were used in the *in vitro* assessments. Mouse liver S9 fraction (Xenotech, female, CD-1, lot# 0310217, 20 mg/mL protein), rat liver S9 fraction (BD Gentest, male Wistar Han, lot# 58237, 20 mg/mL protein), monkey liver S9 (Xenotech, male, cynomolgus, lot# 0210398, 20 mg/mL) and human liver S9 (Celsis, Lot ABT, 20 mg/mL protein) were utilized for the *in vitro* assessments.

3.2. Blood

Mouse, rat, monkey and human blood were spiked with ALC-0159 and ALC-0315 stock solutions (1 mM, each dissolved in DMSO) to give a final concentration of 10 μM. A solvent control was also included where DMSO was added in place of test compound. After addition of test compound or DMSO, blood samples were maintained at 37 °C. Aliquots (500 μL) were removed at 0, 0.5, 1, 2, 4, 6, and 24 h and quenched with 6 volumes of ice-cold MeCN. Samples were subsequently centrifuged at 1860 x g for 5 minutes. The samples were then transferred to clean 15 mL glass tubes and evaporated to dryness using a Genevac evaporative centrifuge. To expedite analyses, only the 0 and 24 h samples were reconstituted

in 100 µL of 1% MeCN in water and analyzed as described below. The remaining samples were stored at -40 °C.

3.3. Hepatocytes

Mouse, rat, monkey and human hepatocyte incubations (0.75 x 10^6 cells/mL), were conducted at a final concentration of ALC-0159 and ALC-0315 of 10 µM using 1 mM stocks, each dissolved in DMSO. A solvent control was also included where DMSO was added in place of test compound. After addition of test compound or DMSO, samples were maintained in an incubator at 37 °C, 95% humidity, and 5% carbon dioxide. Aliquots (500 µL) were removed at 0, 0.5, 1, 2, and 3 h and a 250 µL at 4 h and quenched with 6 volumes of ice-cold MeCN. Samples were subsequently centrifuged at 1860 x g for 5 minutes. The samples were then transferred to clean 15 mL glass tubes and evaporated to dryness using a Genevac evaporative centrifuge. To expedite analyses, only the 0 and 4 h samples were analyzed. The 0 h samples were reconstituted in 100 µL of 1% MeCN in water and 4 h samples in 50 µL of 1% MeCN in water and analyzed as described below. The remaining samples were stored at -40 °C.

3.4. Liver S9 Fractions

Liver S9 fractions from mouse, rat, monkey, and human suspended in 0.1 M phosphate buffer (pH 7.4) containing 3.3 mM magnesium chloride were preincubated with alamethicin (9 µg/mL) for 15 min on ice. Incubations were started by addition of a mixture of test compound dissolved in DMSO and buffer, test compound dissolved in DMSO, buffer, and cofactor mix A (1 mM NADPH, 1 mM NADH, 0.5 mM PAPS, and 2.5 mM UDPGA), or test compound, buffer and cofactor mix B (1 mM NADP+, 1 mM NAD+, 0.5 mM PAPS, and 2.5 mM UDPGA), bringing the incubation to a total volume of 1 mL with a final protein concentration of 1 mg/mL and a final concentration of ALC-0159 or ALC-0315 of 10 µM. Incubation mixtures were warmed to 37 °C, and aliquots (150 µL) were removed at 0, 0.5, 1, 2, 4, 6, and 24 h and quenched by addition to MeCN (400 µL). Samples were subsequently centrifuged at 3000 rpm (1860 x g) for 5 minutes. The samples were then transferred to clean 1 mL glass dolphin-nosed tubes and evaporated to dryness using a Genevac evaporative centrifuge. To expedite analyses, only the 0 and 24 h samples were reconstituted in 100 µL of 1% MeCN in water and analyzed as described below. The remaining samples were stored at -40 °C.

3.5. Rat Pharmacokinetics Study Samples

Plasma, urine, feces and liver samples were obtained from a 14-day rat pharmacokinetics study (Study PF-07302048_06Jul20_072424[1]) where a luciferase-encoding mod RNA formulated in LNP with an identical lipid composition as PF-07302048 (COVID-19 mRNA Vaccine; BioNTech code number BNT162) was administered intravenously at a 1 mg/kg mod RNA dose to male, Wistar Han rats. At this mod RNA dose, the dose of ALC-0159 was 1.96 mg/kg and of ALC-0315 was 15.3 mg/kg. While additional time point samples were obtained of pharmacokinetic analysis, for metabolite identification studies, plasma and livers from three rats per time point at the following time points were used: pre-dose, 0.1, 24, 96, 192, and 336 h post-dose. Fecal and urine samples from three rats per time point from pre-dose, 0-24, 24-48, 72-96, 168-192, and 312-336 h post-dose were used.

3.5.1. Plasma Sample Preparation

Plasma (50 µL) from each rat per time point was pooled to generate pools for the pre-dose, 0.1, 24, 96, 192, and 336 h time points. Proteins were precipitated with 4 volumes of ice-cold MeCN, centrifuged, and the supernatant blown to dryness. Residues were reconstituted in 100 µL of 1% MeCN in water and analyzed as described below.

3.5.2. Urine Sample Preparation

Urine samples (100 µL from each rat) for the pre-dose, 0-24, 24-48, 72-96, 168-192, and 312-336 h time points were combined to generate a sample pool for each of these time points. Pooled urine samples were centrifuged at 17000 x g for 10 minutes. Supernatants were transferred to analyses tubes and analyzed without further manipulation and analyzed as described below.

3.5.3. Feces Sample Preparation

Feces samples for each rat fecal sample from the pre-dose, 0-24, 24-48, 72-96, 168-192, and 312-336 h time points were diluted 1:9 (w/v) with homogenization solution (60:40 isopropyl alcohol/water) and homogenized with a Mini-Beadbeater-96 (BioSpec Products) using 2 mm zirconia beads and a 2 minute homogenization time. Homogenized samples (300 µL) for the three rat samples per time point were pooled. Proteins were precipitated with 4 volumes of ice-cold MeCN, centrifuged, and the supernatant blown to dryness. Residues were reconstituted in 100 µL of 1% MeCN in water and analyzed as described below.

3.5.4. Liver Sample Preparation

Liver samples for each rat from the pre-dose, 0.1, 24, 96, 192, and 336 h time points were diluted 1:4 (w/v) with homogenization solution (60:40 isopropyl alcohol/water) and homogenized with a Mini-Beadbeater-96 (BioSpec Products) using 2 mm zirconia beads and a 2 minute homogenization time. Homogenized samples (200 µL) for the three rat samples per time point were pooled. Proteins were precipitated with 4 volumes of ice-cold MeCN, centrifuged, and the supernatant blown to dryness. Residues were reconstituted in 100 µL of 1% MeCN in water and analyzed as described below.

3.6. UHPLC-MS/MS Analysis

3.6.1. UHPLC-MS/MS Sample Analysis of ALC-0159

Reconstituted samples were analyzed using the same UHPLC method but with separate MS/MS analyses in positive ion and negative ion electrospray modes using a Thermo Orbitrap Elite mass spectrometer. Xcalibur software version 3.0.63 was used to control the UHPLC-MS system. Injections of 2 µL were made by a CTC PAL autosampler. Full scan data were collected at 15,000 resolution. The UHPLC system consisted of an Accela quaternary solvent delivery pump (Thermo Electron Corporation). An Acquity UPLC C8 100 Å column was used (2.1 x 100 mm, 1.7 µm) with a flow rate of 0.4 mL/min heated to 45 °C in a Hot Sleeve column heater (Analytical Sales and Services). Mobile phase A was 10 mM ammonium acetate buffer (pH 4.5) and mobile phase B was MeCN.

Time, min	%A	%B	Flow Rate (µL/min)
0.0	100	0	400
2.5	100	0	400
5.0	40	60	400
23.0	5	95	400
26.0	5	95	400
26.1	100	0	400
30.0	100	0	400

3.6.2. UHPLC-MS/MS Sample Analysis of ALC-0315

Reconstituted samples were analyzed using the same UHPLC method but with separate MS/MS analyses in positive ion and negative ion electrospray modes using a Thermo Orbitrap Elite mass spectrometer. Xcalibur software version 3.0.63 was used to control the UHPLC-MS system. Injections of 5 µL were made by a CTC PAL autosampler. Full scan data were collected at 15,000 resolution. The UHPLC system consisted of an Accela quaternary solvent delivery pump (Thermo Electron Corporation). An Acquity UPLC C18 100 Å column was used (2.1 x 150 mm, 1.7 µm) with a flow rate of 0.3 mL/min heated to 45 °C in a Hot Sleeve column heater (Analytical Sales and Services). Mobile phase A was 0.1 % formic acid in water and mobile phase B was MeCN.

Time, min	%A	%B	Flow Rate (µL/min)
0.0	100	0	300
2.5	100	0	300
5.0	90	10	300
10.0	50	50	300
17.5	5	95	300
21.5	5	95	300
21.6	100	0	300
25.0	100	0	300

4. RESULTS & DISCUSSION

As shown in Figure 9.1, the primary route of metabolism identified for ALC-0159 involves amide bond hydrolysis yielding *N*,*N*-ditetradecylamine (*m/z* 410). This metabolite was identified in mouse and rat blood as well as hepatocytes and liver S9 from mouse, rat, monkey and human. Theoretical metabolites were arrived at via examination of the excipient molecules and consideration of commonly observed biotransformations (hydroxylation, *N*-dealkylation, hydrolysis, glucuronidation, sulfation, oxidation and combinations thereof). Summaries of the masses of theoretical and observed metabolites of ALC-0159 for blood, hepatocytes, liver S9 fractions, and rat pharmacokinetics samples are presented in Tables 8.1, 8.2, 8.3, and 8.4, respectively. Representative example chromatograms from *in vitro* incubations of ALC-0159 with mouse hepatocytes, human hepatocytes, and *in vivo* samples from a rat pharmacokinetics study are presented in Figures 9.3, 9.4, and 9.5, respectively. No metabolites of ALC-0159 were identified from *in vivo* samples.

Metabolism of ALC-0315 occurs via two sequential ester hydrolysis reactions, first yielding the monoester metabolite (*m/z* 528) followed by the doubly deesterified metabolite (*m/z* 290) as shown in Figure 9.2. The monoester metabolite was observed in vitro in rat blood, monkey S9 fraction, and *in vivo* in rat plasma and rat liver. The doubly deesterified metabolite was observed *in vitro* in mouse and rat blood; monkey liver S9 fraction; and *in vivo* in rat plasma, urine, feces and liver. Subsequent metabolism of the doubly desterified metabolite resulted in a glucuronide metabolite (*m/z* 466) which was observed in urine only from the rat pharmacokinetics study. Additionally, 6-hexyldecanoic acid (*m/z* 255), the acid product of both hydrolysis reactions of ALC-0315, was identified *in vitro* in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma. Summaries of the masses of theoretical and observed metabolites of ALC-0315 for blood, hepatocytes, liver S9 fractions, and rat pharmacokinetics samples are presented in Tables 8.5, 8.6, 8.7, and 8.8, respectively. Representative example chromatograms from *in vitro* incubations of ALC-03159 with monkey liver S9 fraction, human hepatocytes, and *in vivo* samples from a rat pharmacokinetics study are presented in Figures 9.6, 9.7, and 9.8, respectively.

Based on nonquantitative ion current data for parent depletion and metabolite formation, metabolism for both ALC-0159 and ALC-0315 appears to occur relatively slowly across most species *in vitro* and *in vivo*. Overall, it can be concluded that both ALC-0159 and ALC-0315 are metabolized by hydrolytic metabolism of the amide or ester functionalities, respectively, and this hydrolytic metabolism is observed across the species evaluated.

4.1. Mass Spectral Analysis of ALC-0159

Mass spectrometric analyses of ALC-0159 indicate that it is a mixture of varying polyethylene glycol (PEG) lengths ranging between approximately 40-51 ethylene glycol units. Additionally, the mass spectrum (Figure 9.9) indicates that this mixture of compounds also exists in +2, +3, and +4 charge states with the +4 charge state being the most abundant. Deconvolution of the most abundant ion in the +4 charge state (*m/z* 629.6939, t_R = 19.1 minutes) is consistent with a triply ammoniated, protonated species. For simplicity of analyses and description, PEG-containing metabolites of ALC-0159, where standards are not available, were searched for based on modifications of the most abundant and intense parent mass (*m/z* 629.6939).

4.2. Mass Spectral Analysis of ALC-0159 *m/z* 410 metabolite

An *m/z* 410 metabolite of ALC-0159 had a retention time of approximately 16.9 minutes with a protonated molecular ion of *m/z* 410.4715. It was observed in mouse and rat blood, as well as hepatocytes and liver S9 from mouse, rat, monkey and human. The product ion spectrum for *m/z* 410 possessed a single fragment ion of *m/z* 214 which corresponds to loss of one of the 14-carbon aliphatic chains. Both the observed retention time and fragmentation pattern for *m/z* 410 match those obtained from *N*,*N*-ditetradecylamine (Figure 9.10)

4.3. Mass Spectral Analysis of ALC-0315

ALC-0315 was identified at a retention time of 20.0 minutes and *m/z* 766.7254. Product ion spectrum fragment ions at *m/z* 748, 694 and 510 were observed in as shown in Figure 9.11. The *m/z* 748 fragment is consistent with a water loss from butyl alcohol substituent. The *m/z*

694 fragment corresponds to loss of the butyl alcohol substituent. The *m/z* 272 fragment is consistent with loss of one of the 6-hexyldecanoic acid moieties along with H+.

4.4. Mass Spectral Analysis of ALC-0315 *m/z* 528 Metabolite

A metabolite of ALC-0315 with a retention time of 15.9 minutes and *m/z* 528.4975. This metabolite was observed in rat blood, monkey liver S9 fraction, rat plasma and rat liver samples. Product ion spectrum fragment ions at *m/z* 510, 456, 272 and 218 were observed as shown in Figure 9.12. The *m/z* 510 fragment is consistent with a water loss from one of the two alkyl alcohol substituents. The *m/z* 456 fragment corresponds to loss of the butyl alcohol substituent. The *m/z* 272 fragment is consistent with loss of the 6-hexyldecanoic acid moiety along with H+.

4.5. Mass Spectral Analysis of ALC-0315 *m/z* 290 Metabolite

A metabolite of ALC-0315 was observed at 8.0 minutes with *m/z* 290.2688. This metabolite was observed in mouse and rat blood, monkey liver S9 fraction, and plasma, urine, feces and liver from the rat pharmacokinetics study. The product ion spectrum displays fragment ions of *m/z* 272 (loss of water) and *m/z* 218 (loss of butyl alcohol substituent) (Figure 9.13).

4.6. Mass Spectral Analysis of ALC-0315 *m/z* 466 Metabolite

The *m/z* 466 metabolite of ALC-0315 was observed at 7.9 minutes with *m/z* 466.3006 only in rat urine. The product ion spectra of shows a single fragment with *m/z* 290 (Figure 9.14). A neutral loss of 176 Da for this metabolite is consistent with a glucuronide conjugate to one of the three alcohol moieties of the doubly deesterified metabolite, *m/z* 290.

4.7. Mass Spectral Analysis of ALC-0315 *m/z* 255 Metabolite

An *m/z* 255 metabolite of ALC-0315 was observed at approximately 19.7 min with *m/z* 255.2324 in mouse plasma. This metabolite was observed in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma. This metabolite matches by both retention time and exact mass with a synthetic standard of 6-hexyldecanoic acid. However, product ion spectra could not be obtained for either the metabolite or the 6-hexyldecanoic acid standard.

5. CONCLUSIONS

The metabolism of the novel excipients, ALC-0159 and ALC-0315, were examined *in vitro* using blood, liver S9 fractions and hepatocytes, all from mouse, rat, monkey and human. The *in vivo* metabolism was examined in rat plasma, urine, feces, and liver from a rat pharmacokinetics study where a luciferase-encoding mod RNA formulated in an LNP with an identical lipid composition as PF-07302048 was administered intravenously at a 1 mg/kg dose.

The primary route of metabolism identified for ALC-0159 involves amide bond hydrolysis yielding *N*,*N*-ditetradecylamine (*m/z* 410). This metabolite was identified in mouse and rat blood, as well as hepatocytes and liver S9 from mouse, rat, monkey and human. No metabolites of ALC-0159 were identified from *in vivo* samples.

Metabolism of ALC-0315 occurs via two sequential ester hydrolysis reactions, first yielding the monoester metabolite (*m/z* 528) followed by the doubly deesterified metabolite (*m/z* 290). The monoester metabolite was observed *in vitro* in rat blood, monkey S9 fraction, and *in vivo* in rat plasma and rat liver. The doubly deesterified metabolite was observed *in vitro* in mouse and rat blood; monkey liver S9 fraction; and *in vivo* in rat plasma, urine, feces and liver. Subsequent metabolism of the doubly deesterified metabolite resulted in a glucuronide metabolite (*m/z* 466) which was observed in urine only from the rat pharmacokinetics study. Additionally, 6-hexyldecanoic acid (*m/z* 255), the acid product of both hydrolysis reactions of ALC-0315, was identified *in vitro* in mouse and rat blood; mouse, rat, monkey and human hepatocytes; mouse, rat and human liver S9 fractions; and *in vivo* in rat plasma.

Based on nonquantitative ion current data for parent depletion and metabolite formation, metabolism for both ALC-0159 and ALC-0315 appears to occur relatively slowly across most species *in vitro* and *in vivo*. Overall, it can be concluded that both ALC-0159 and ALC-0315 are metabolized by hydrolytic metabolism of the amide or ester functionalities, respectively, and this hydrolytic metabolism is observed across the species evaluated.

6. ARCHIVING

Data presented in this report can be found in the following locations:

Laboratory Notebooks	/Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200715_COVID_Novel_Excipients_HHEP /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200728_COVID_Novel_Excipients_Blood_Stability /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200728_COVID_Novel_Excipients_S9 /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200820_COVID_excipient_rat_PK_met_ID

Laboratory Notebooks	/Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200715_COVID_Novel_Excipients_HHEP /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200728_COVID_Novel_Excipients_Blood_Stability /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200728_COVID_Novel_Excipients_S9 /Root/PDM/Groton/ (b) (6) VBN#00710777/COVID_Excipient/200820_COVID_excipient_rat_PK_met_ID
Analytical Archive Reference	Open Lab: Enterprise/Content/Target Archive/ COVID vaccine excipients/Biotransformation/ 200730_COVID_Excipient_HEP Enterprise/Content/Target Archive/ COVID vaccine excipients/Biotransformation/ 200730_COVID_Excipients_LS9 Enterprise/Content/Target Archive/ COVID vaccine excipients/Biotransformation/ 200731_COVID_Excipient_Blood Enterprise/Content/Target Archive/ COVID vaccine excipients/Biotransformation/ 200822_COVID_Excipient_Rat_PK

7. REFERENCES

1. PF-07302048_06Jul20_072424_A single dose pharmacokinetics study of ALC-0315 and ALC-0159 following intravenous bolus injection of PF-07302048 Nanoparticle Formulation in Wistar Han Rats. 01 Sept 2020.

8. SUPPORTIVE TABLES

8.1. *In Vitro* Assessment of ALC-0159 Metabolites in Mouse, Rat, Monkey and Human Blood

m/z	Biotransformation	t_R, min	Blood			
			Mouse	Rat	Monkey	Human
107.0703[b]	*O*-Demethylation, *O*-dealkylation	1.2[c]	ND	ND	ND	ND
151.0965[b]	*O*-Demethylation, *O*-dealkylation	3.1[c]	ND	ND	ND	ND
195.1227[b]	*O*-Demethylation, *O*-dealkylation	4.8[c]	ND	ND	ND	ND
214.2529[b]	Hydrolysis, *N*-dealkylation	7.3[c]	ND	ND	ND	ND
227.2017[a]	*N*-Dealkylation, oxidation	9.1[c]	ND	ND	ND	ND
410.4720[b]	Hydrolysis (amine)	16.9[c]	+	+	ND	ND
531.5849[b]	*N*,*N*-Didealkylation	ND	ND	ND	ND	ND
580.6396[b]	*N*-Dealkylation	ND	ND	ND	ND	ND
629.6853[b]	*O*-Demethylation, oxidation	ND	ND	ND	ND	ND
633.6931[b]	Hydroxylation	ND	ND	ND	ND	ND
637.1880[b]	ω-Hydroxylation, oxidation	ND	ND	ND	ND	ND
708.7721[b]	Hydrolysis (acid)	5.8[c]	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.2. *In Vitro* Assessment of ALC-0159 Metabolites in Mouse, Rat, Monkey and Human Hepatocytes

m/z	Biotransformation	t_R, min	Hepatocytes			
			Mouse	Rat	Monkey	Human
107.0703[b]	*O*-Demethylation, *O*-dealkylation	1.2[c]	ND	ND	ND	ND
151.0965[b]	*O*-Demethylation, *O*-dealkylation	3.1[c]	ND	ND	ND	ND
195.1227[b]	*O*-Demethylation, *O*-dealkylation	4.8[c]	ND	ND	ND	ND
214.2529[b]	Hydrolysis, *N*-dealkylation	7.3[c]	ND	ND	ND	ND
227.2017[a]	*N*-Dealkylation, oxidation	9.1[c]	ND	ND	ND	ND
410.4720[b]	Hydrolysis (amine)	16.9[c]	+	+	+	+
531.5849[b]	*N*,*N*-Didealkylation	ND	ND	ND	ND	ND
580.6396[b]	*N*-Dealkylation	ND	ND	ND	ND	ND
629.6853[b]	*O*-Demethylation, oxidation	ND	ND	ND	ND	ND
633.6931[b]	Hydroxylation	ND	ND	ND	ND	ND
637.1880[b]	ω-Hydroxylation, oxidation	ND	ND	ND	ND	ND
708.7721[b]	Hydrolysis (acid)	5.8[c]	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.3. *In Vitro* Assessment of ALC-0159 Metabolites in Mouse, Rat, Monkey and Human Liver S9 Fractions

m/z	Biotransformation	t_R, min	Liver S9 Fractions			
			Mouse	Rat	Monkey	Human
107.0703[b]	*O*-Demethylation, *O*-dealkylation	1.2[c]	ND	ND	ND	ND
151.0965[b]	*O*-Demethylation, *O*-dealkylation	3.1[c]	ND	ND	ND	ND
195.1227[b]	*O*-Demethylation, *O*-dealkylation	4.8[c]	ND	ND	ND	ND
214.2529[b]	Hydrolysis, *N*-dealkylation	7.3[c]	ND	ND	ND	ND
227.2017[a]	*N*-Dealkylation, oxidation	9.1[c]	ND	ND	ND	ND
410.4720[b]	Hydrolysis (amine)	16.9[c]	+	+	+	+
531.5849[b]	*N*,*N*-Didealkylation	ND	ND	ND	ND	ND
580.6396[b]	*N*-Dealkylation	ND	ND	ND	ND	ND
629.6853[b]	*O*-Demethylation, oxidation	ND	ND	ND	ND	ND
633.6931[b]	Hydroxylation	ND	ND	ND	ND	ND
637.1880[b]	ω-Hydroxylation, oxidation	ND	ND	ND	ND	ND
708.7721[b]	Hydrolysis (acid)	5.8[c]	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.4. Assessment of Metabolites of ALC-0159 in Plasma, Urine, Feces, and Liver from a Rat Pharmacokinetics Study.

m/z	Biotransformation	t_R, min	Rat *In Vivo*			
			Plasma	Urine	Feces	Liver
107.0703[b]	*O*-Demethylation, *O*-dealkylation	1.2[c]	ND	ND	ND	ND
151.0965[b]	*O*-Demethylation, *O*-dealkylation	3.1[c]	ND	ND	ND	ND
195.1227[b]	*O*-Demethylation, *O*-dealkylation	4.8[c]	ND	ND	ND	ND
214.2529[b]	Hydrolysis, *N*-dealkylation	7.3[c]	ND	ND	ND	ND
227.2017[a]	*N*-Dealkylation, oxidation	9.1[c]	ND	ND	ND	ND
410.4720[b]	Hydrolysis (amine)	16.9[c]	ND	ND	ND	ND
531.5849[b]	*N*,*N*-Didealkylation	ND	ND	ND	ND	ND
580.6396[b]	*N*-Dealkylation	ND	ND	ND	ND	ND
629.6853[b]	*O*-Demethylation, oxidation	ND	ND	ND	ND	ND
633.6931[b]	Hydroxylation	ND	ND	ND	ND	ND
637.1880[b]	ω-Hydroxylation, oxidation	ND	ND	ND	ND	ND
708.7721[b]	Hydrolysis (acid)	5.8[c]	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.5. *In Vitro* Assessment of ALC-0315 Metabolites in Mouse, Rat, Monkey and Human Blood

m/z	Biotransformation	t_R, min	Blood			
			Mouse	Rat	Monkey	Human
102.0561[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
104.0706[b]	*N*-Dealkylation, oxidation	1.2[c]	ND	ND	ND	ND
130.0874[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
132.1019[b]	*N*-Dealkylation, oxidation	1.9[c]	ND	ND	ND	ND
145.0506[a]	*N*-Dealkylation, hydrolysis, oxidation	7.7[c]	ND	ND	ND	ND
255.2330[a]	Hydrolysis (acid)	19.7[c]	+	+	ND	ND
271.2279[a]	Hydrolysis, hydroxylation	ND	ND	ND	ND	ND
290.2690[b]	Bis-hydrolysis (amine)	8.1	+	+	ND	ND
431.2650[a]	Hydrolysis, glucuronidation	ND	ND	ND	ND	ND
464.2865[a]	Bis-hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
466.3011[b]	Bis-hydrolysis (amine), glucuronidation	7.9	ND	ND	ND	ND
528.4986[b]	Hydrolysis (amine)	15.9	ND	+	ND	ND
704.5307[b]	Hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
778.6930[a]	Oxidation to acid	ND	ND	ND	ND	ND
780.7076[b]	Oxidation to acid	ND	ND	ND	ND	ND
782.7232[b]	Hydroxylation	ND	ND	ND	ND	ND
844.6706[a]	Sulfation	ND	ND	ND	ND	ND
846.6851[b]	Sulfation	ND	ND	ND	ND	ND
940.7458[a]	Glucuronidation	ND	ND	ND	ND	ND
942.7604[b]	Glucuronidation	ND	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.6. *In Vitro* Assessment of ALC-0315 Metabolites in Mouse, Rat, Monkey and Human Hepatocytes

m/z	Biotransformation	t_R, min	Hepatocytes			
			Mouse	Rat	Monkey	Human
102.0561[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
104.0706[b]	*N*-Dealkylation, oxidation	1.2[c]	ND	ND	ND	ND
130.0874[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
132.1019[b]	*N*-Dealkylation, oxidation	1.9[c]	ND	ND	ND	ND
145.0506[a]	*N*-Dealkylation, hydrolysis, oxidation	7.7[c]	ND	ND	ND	ND
255.2330[a]	Hydrolysis (acid)	19.7[c]	+	+	+	+
271.2279[a]	Hydrolysis, hydroxylation	ND	ND	ND	ND	ND
290.2690[b]	Bis-hydrolysis (amine)	8.1	ND	ND	ND	ND
431.2650[a]	Hydrolysis, glucuronidation	ND	ND	ND	ND	ND
464.2865[a]	Bis-hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
466.3011[b]	Bis-hydrolysis (amine), glucuronidation	7.9	ND	ND	ND	ND
528.4986[b]	Hydrolysis (amine)	15.9	ND	ND	ND	ND
704.5307[b]	Hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
778.6930[a]	Oxidation to acid	ND	ND	ND	ND	ND
780.7076[b]	Oxidation to acid	ND	ND	ND	ND	ND
782.7232[b]	Hydroxylation	ND	ND	ND	ND	ND
844.6706[a]	Sulfation	ND	ND	ND	ND	ND
846.6851[b]	Sulfation	ND	ND	ND	ND	ND
940.7458[a]	Glucuronidation	ND	ND	ND	ND	ND
942.7604[b]	Glucuronidation	ND	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

8.7. *In Vitro* Assessment of ALC-0315 Metabolites in Mouse, Rat, Monkey and Human Liver S9 Fractions

m/z	Biotransformation	t_R, min	Liver S9 Fractions			
			Mouse	Rat	Monkey	Human
102.0561[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
104.0706[b]	*N*-Dealkylation, oxidation	1.2[c]	ND	ND	ND	ND
130.0874[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
132.1019[b]	*N*-Dealkylation, oxidation	1.9[c]	ND	ND	ND	ND
145.0506[a]	*N*-Dealkylation, hydrolysis, oxidation	7.7[c]	ND	ND	ND	ND
255.2330[a]	Hydrolysis (acid)	19.7[c]	+	+	ND	+
271.2279[a]	Hydrolysis, hydroxylation	ND	ND	ND	ND	ND
290.2690[b]	Bis-hydrolysis (amine)	8.1	ND	ND	+	ND
431.2650[a]	Hydrolysis, glucuronidation	ND	ND	ND	ND	ND
464.2865[a]	Bis-hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
466.3011[b]	Bis-hydrolysis (amine), glucuronidation	7.9	ND	ND	ND	ND
528.4986[b]	Hydrolysis (amine)	15.9	ND	ND	+	ND
704.5307[b]	Hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
778.6930[a]	Oxidation to acid	ND	ND	ND	ND	ND
780.7076[b]	Oxidation to acid	ND	ND	ND	ND	ND
782.7232[b]	Hydroxylation	ND	ND	ND	ND	ND
844.6706[a]	Sulfation	ND	ND	ND	ND	ND
846.6851[b]	Sulfation	ND	ND	ND	ND	ND
940.7458[a]	Glucuronidation	ND	ND	ND	ND	ND
942.7604[b]	Glucuronidation	ND	ND	ND	ND	ND

a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

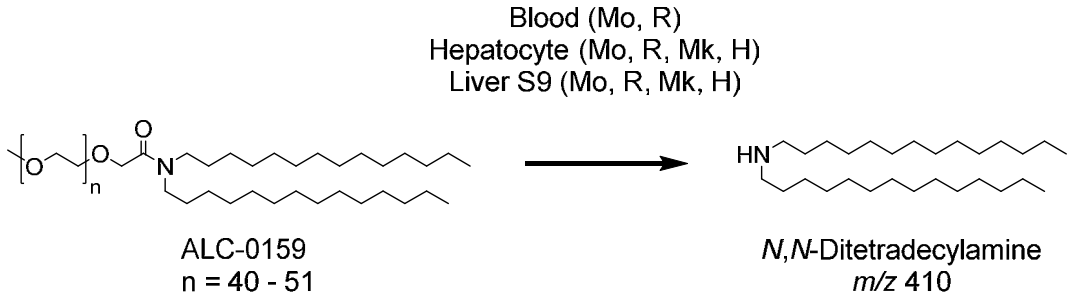
8.8. Assessment of Metabolites of ALC-0315 in Plasma, Urine, Feces, and Liver from a Rat Pharmacokinetics Study.

m/z	Biotransformation	t_R, min	Rat			
			Plasma	Urine	Feces	Liver
102.0561[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
104.0706[b]	*N*-Dealkylation, oxidation	1.2[c]	ND	ND	ND	ND
130.0874[a]	*N*-Dealkylation, oxidation	ND	ND	ND	ND	ND
132.1019[b]	*N*-Dealkylation, oxidation	1.9[c]	ND	ND	ND	ND
145.0506[a]	*N*-Dealkylation, hydrolysis, oxidation	7.7[c]	ND	ND	ND	ND
255.2330[a]	Hydrolysis (acid)	19.7[c]	+	ND	ND	ND
271.2279[a]	Hydrolysis, hydroxylation	ND	ND	ND	ND	ND
290.2690[b]	Bis-hydrolysis (amine)	8.1	+	+	+	+
431.2650[a]	Hydrolysis, glucuronidation	ND	ND	ND	ND	ND
464.2865[a]	Bis-hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
466.3011[b]	Bis-hydrolysis (amine), glucuronidation	7.9	ND	+	ND	ND
528.4986[b]	Hydrolysis (amine)	15.9	+	ND	ND	+
704.5307[b]	Hydrolysis (amine), glucuronidation	ND	ND	ND	ND	ND
778.6930[a]	Oxidation to acid	ND	ND	ND	ND	ND
780.7076[b]	Oxidation to acid	ND	ND	ND	ND	ND
782.7232[b]	Hydroxylation	ND	ND	ND	ND	ND
844.6706[a]	Sulfation	ND	ND	ND	ND	ND
846.6851[b]	Sulfation	ND	ND	ND	ND	ND
940.7458[a]	Glucuronidation	ND	ND	ND	ND	ND
942.7604[b]	Glucuronidation	ND	ND	ND	ND	ND

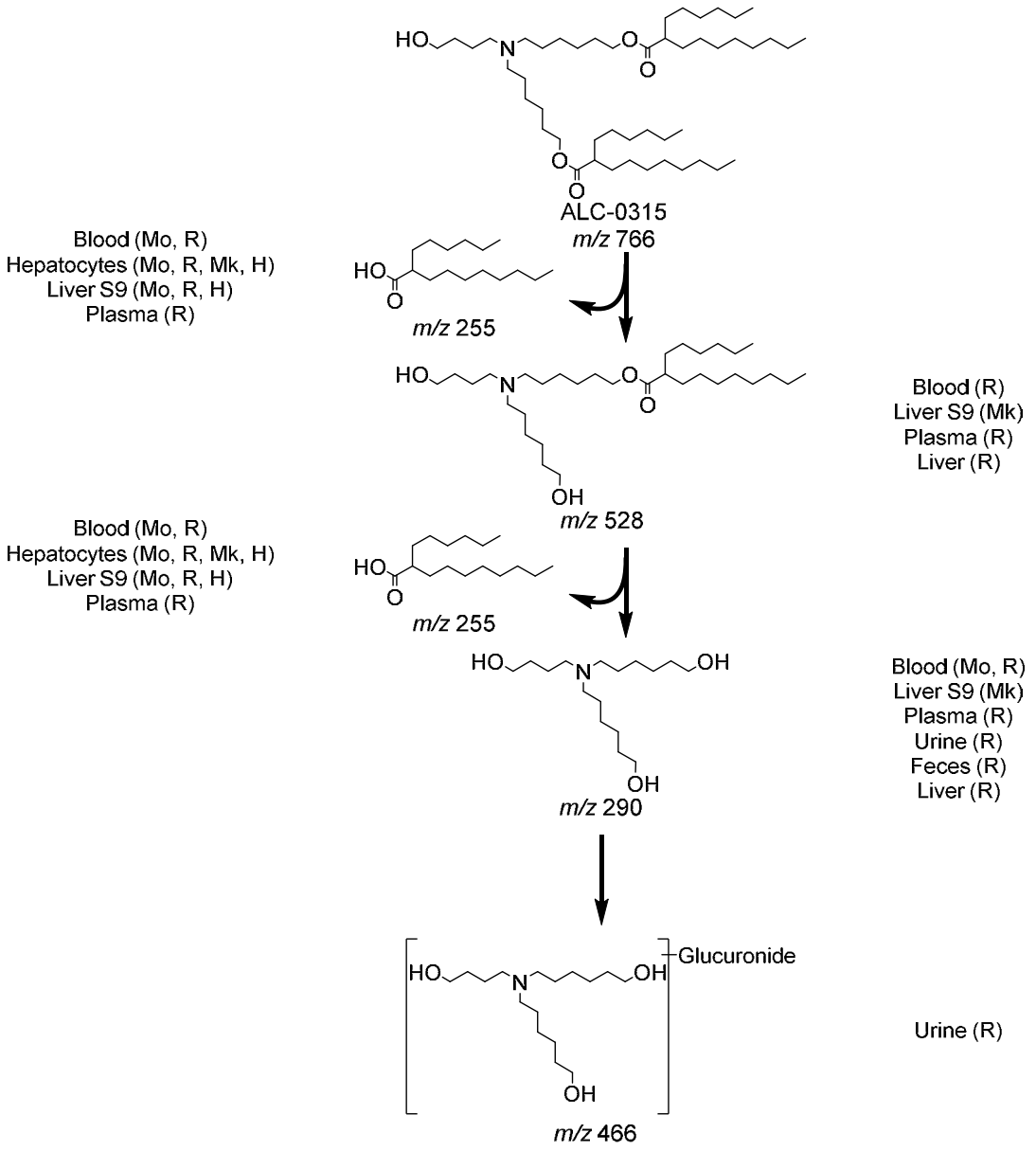
a. Negative ion mode
b. Positive ion mode
c. Determined using standard
ND – Not Detected, + = metabolite identified.

9. SUPPORTIVE FIGURES

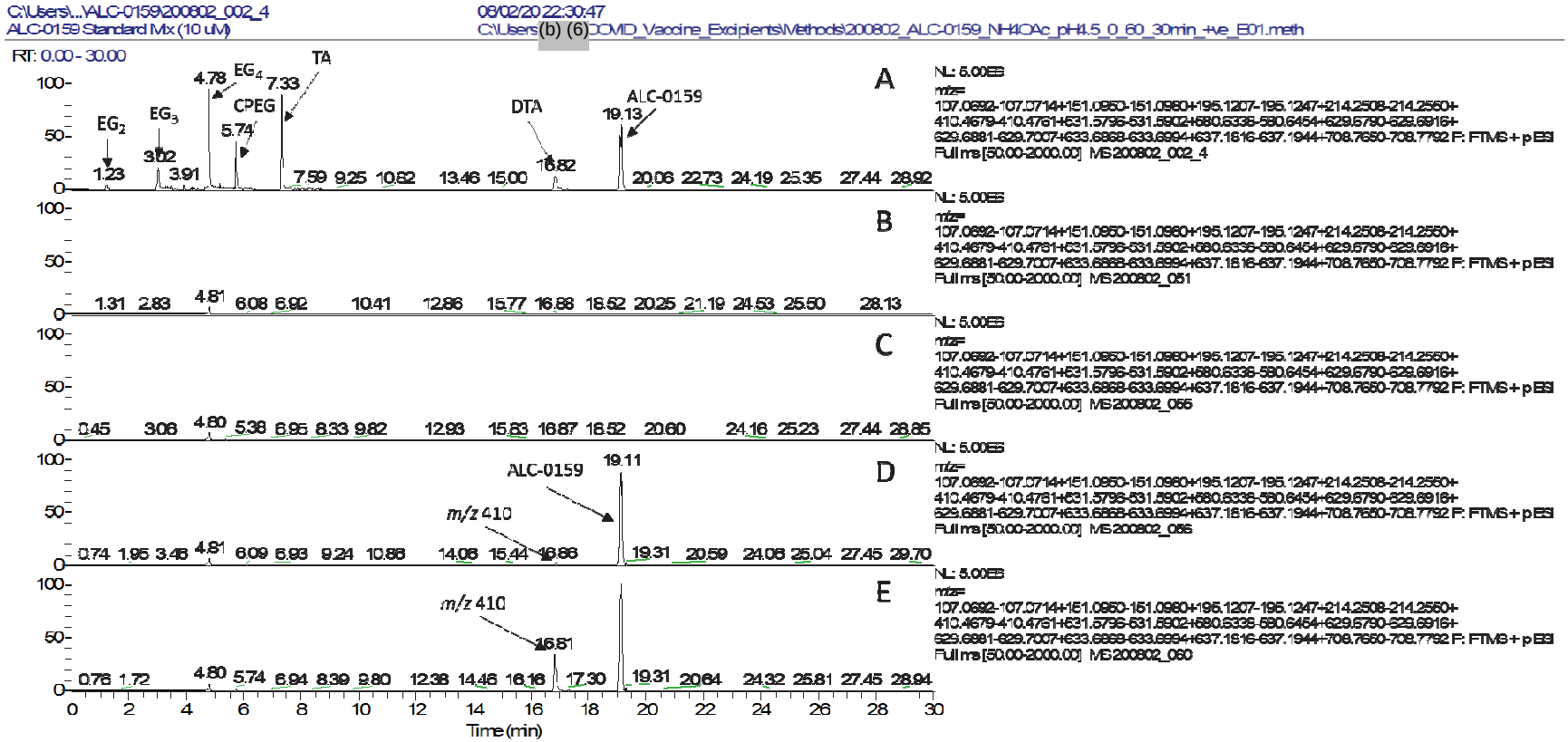
9.1. Proposed Biotransformation Pathway of ALC-0159 in Mouse (Mo), Rat (R), Monkey (Mk) and Human (H)



9.2. Proposed Biotransformation Pathway of ALC-0315 in Mouse (Mo), Rat (R), Monkey (Mk) and Human (H)

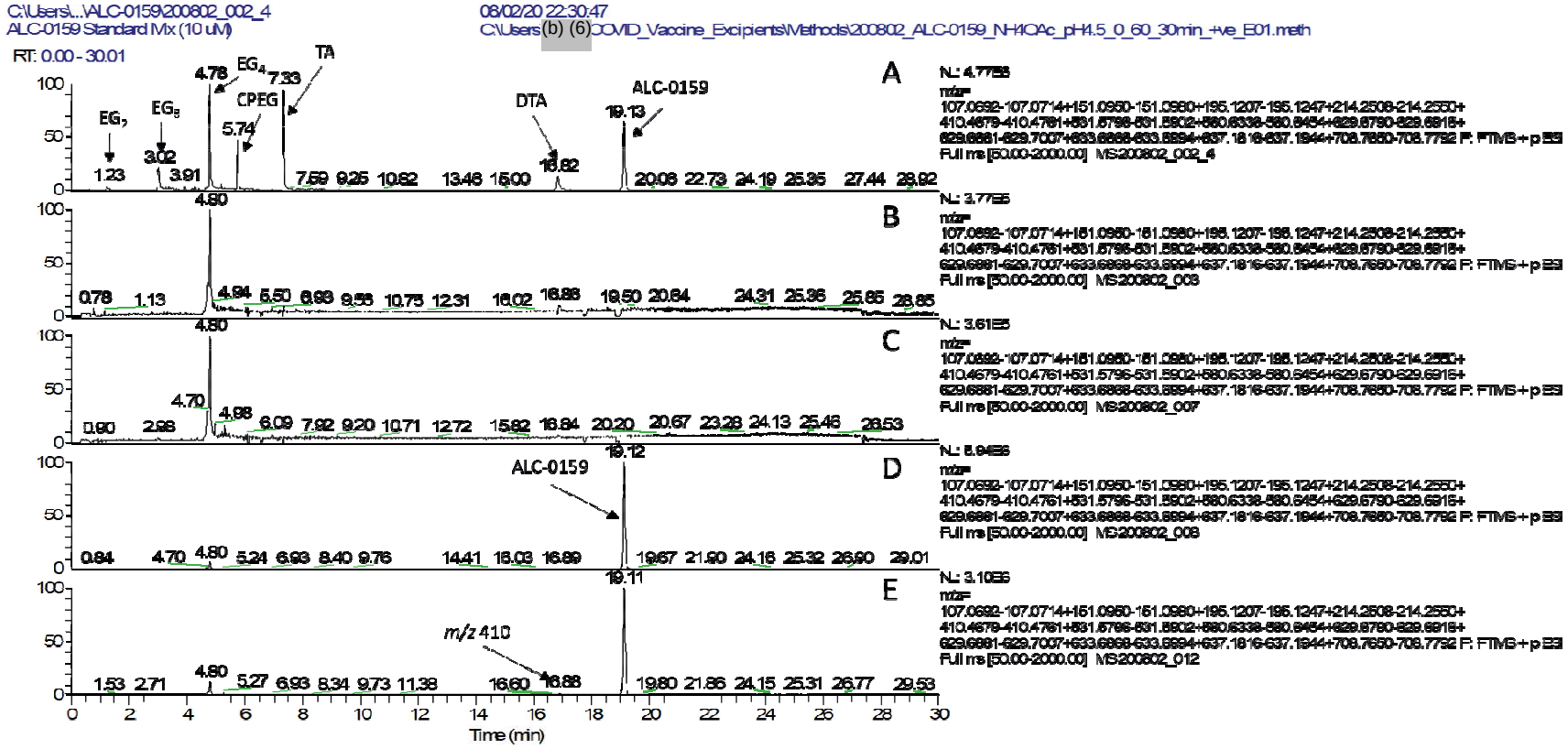


9.3. UHPLC-MS Chromatograms of Standards (A), Blank Mouse Hepatocytes 0 h (B), Blank Mouse Hepatocytes 4 h (C), ALC-0159 in Mouse Hepatocytes 0 h (D) and ALC-0159 in Mouse Hepatocytes 4 h (E)



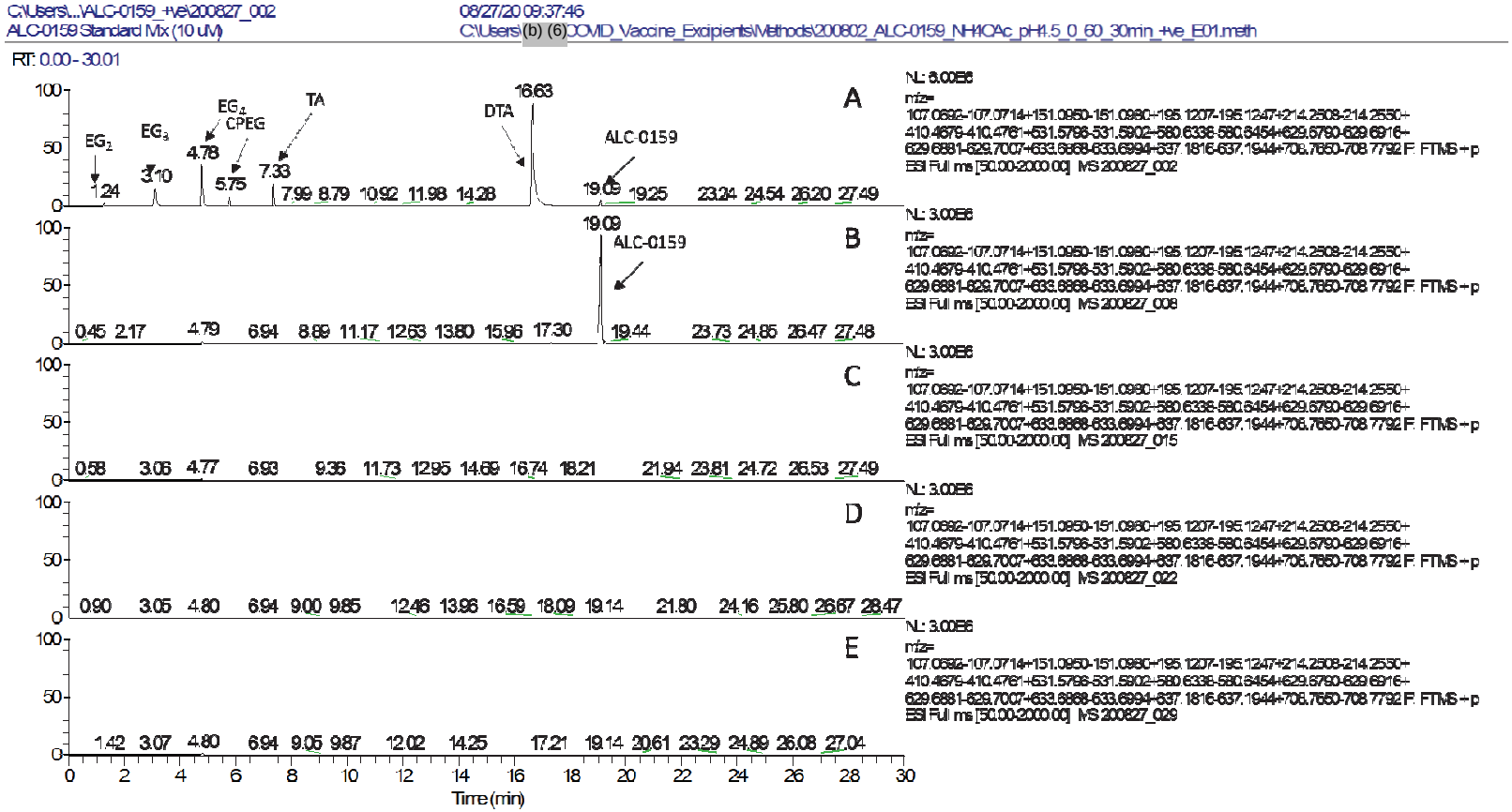
EG_2 – Diethylene glycol
EG_3 – Triethylene glycol
EG_4 – Tetraethylene glycol
CPEG – Carboxy-MPEG2
TA – Tetradecylamine
DTA – *N,N*-Ditetradecylamine

9.4. UHPLC-MS Chromatogram of Standards (A), Blank Human Hepatocytes 0 h (B), Blank Human Hepatocytes 4 h (C), ALC-0159 in Human Hepatocytes 0 h (D) and ALC-0159 in Human Hepatocytes 4 h (E)



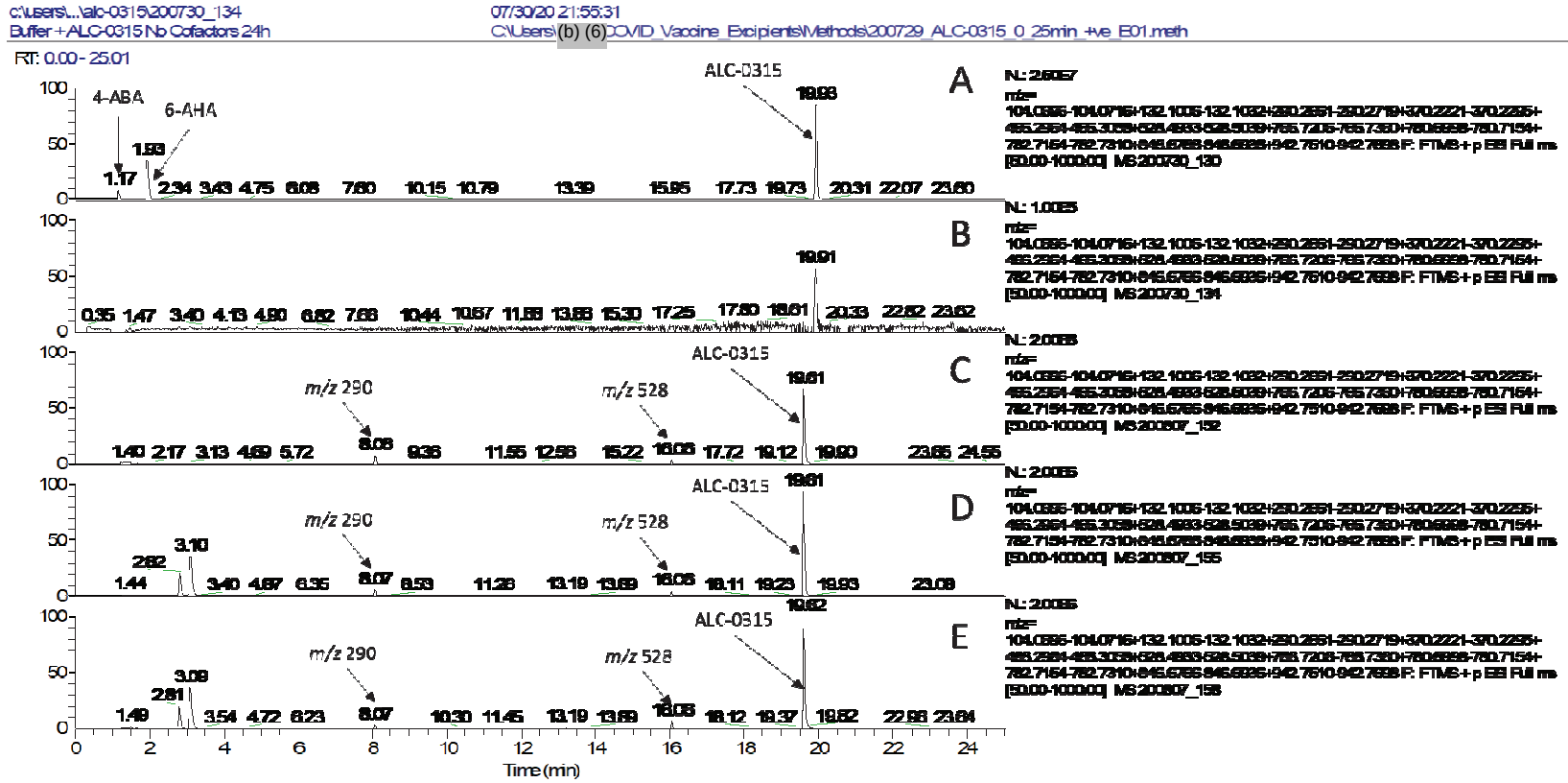
EG_2 – Diethylene glycol
EG_3 – Triethylene glycol
EG_4 – Tetraethylene glycol
CPEG – Carboxy-MPEG2
TA – Tetradecylamine
DTA – *N,N*-Ditetradecylamine

9.5. UHPLC-MS Chromatogram Standards (A), ALC-0159 in Plasma (B), Urine (C), Feces (D) and Liver (E) from a Rat Pharmacokinetics Study



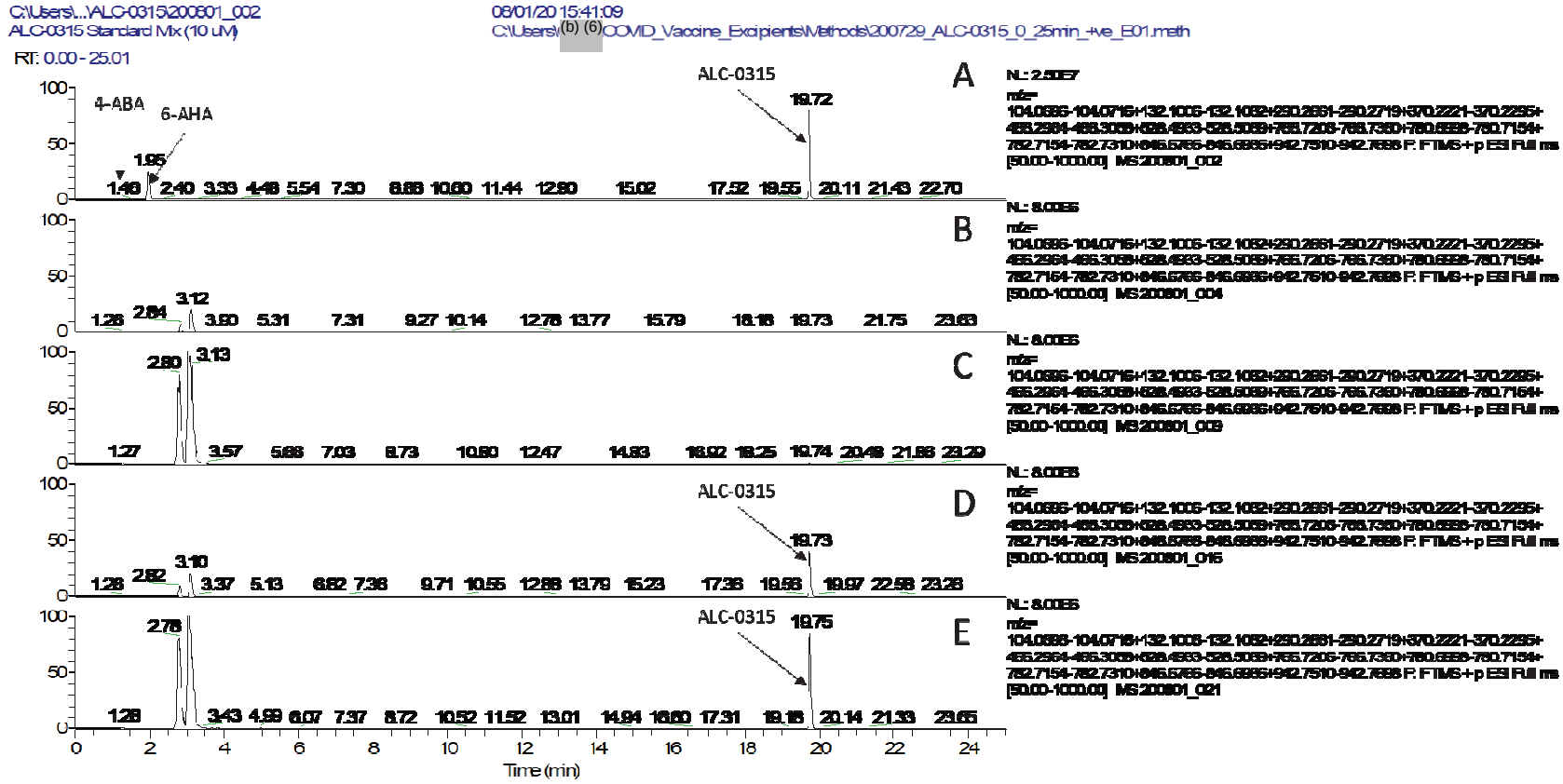
EG_2 – Diethylene glycol
EG_3 – Triethylene glycol
EG_4 – Tetraethylene glycol
CPEG – Carboxy-MPEG2
TA – Tetradecylamine
DTA – *N,N*-Ditetradecylamine

9.6. UHPLC-MS Chromatogram Standards (A), ALC-0315 in buffer (B), ALC-0315 + Monkey Liver S9 No Cofactors 24 h (C), ALC-0315 + Monkey Liver S9 Cofactors Mix A 24 h (D) and ALC-0315 + Monkey Liver S9 Cofactors Mix B 24 h (E)



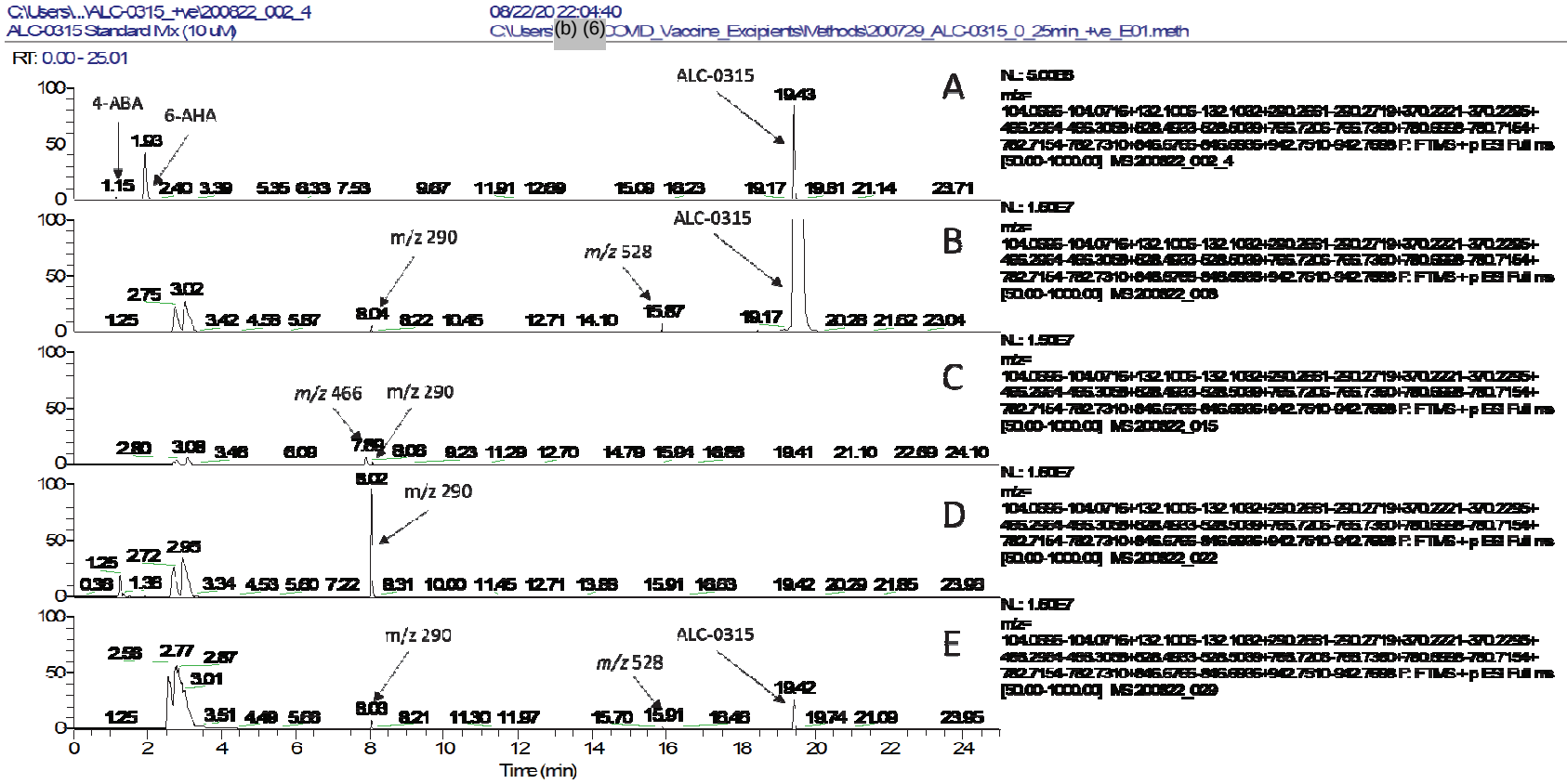
4-ABA – 4-Aminobutyric acid
6-AHA – 6-Aminohexanoic acid

9.7. UHPLC-MS Chromatogram Standards (A), Blank Human Hepatocytes 0 h (B), Blank Human Hepatocytes 4 h (C), ALC-0315 in Human Hepatocytes 0 h (D) and ALC-0315 in Human Hepatocytes 4 h (E)



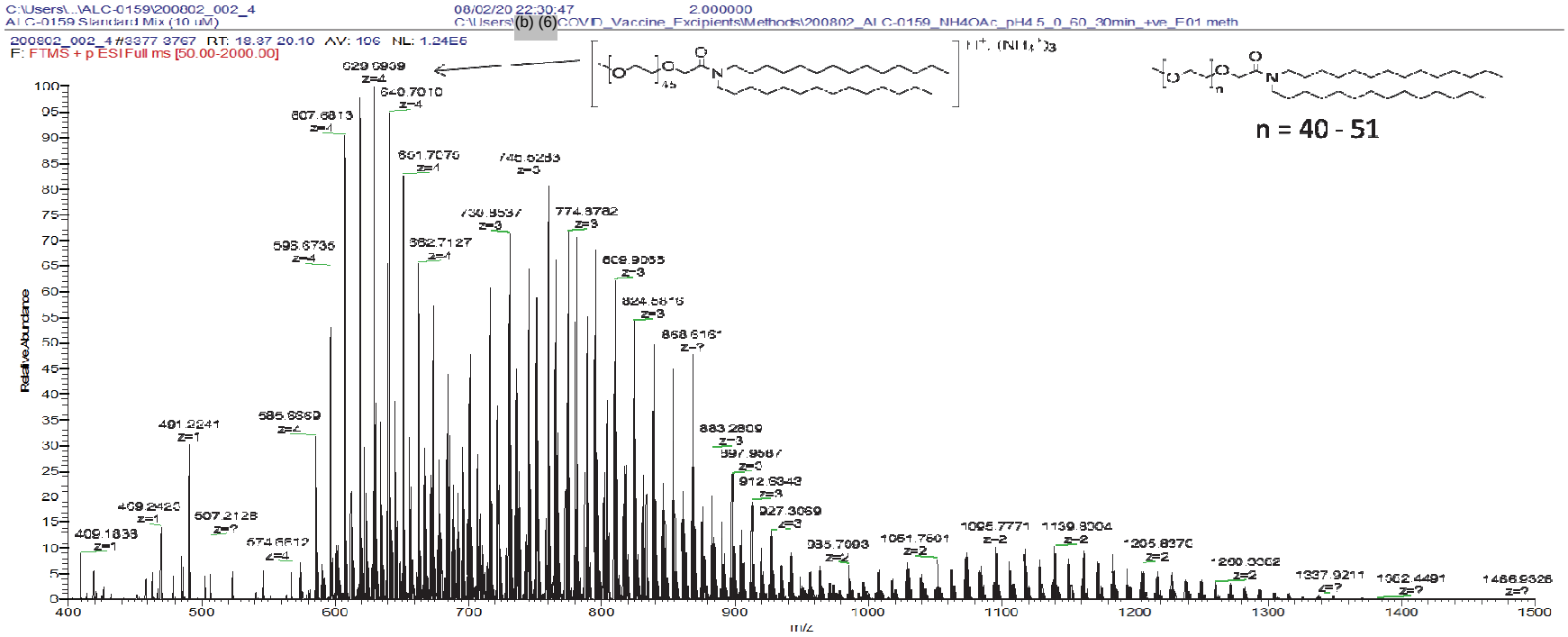
4-ABA – 4-Aminobutyric acid
6-AHA – 6-Aminohexanoic acid

9.8. UHPLC-MS Chromatogram for Standards (A), ALC-0159 in Plasma (B), Urine (C), Feces (D) and Liver (E) from a Rat Pharmacokinetics Study

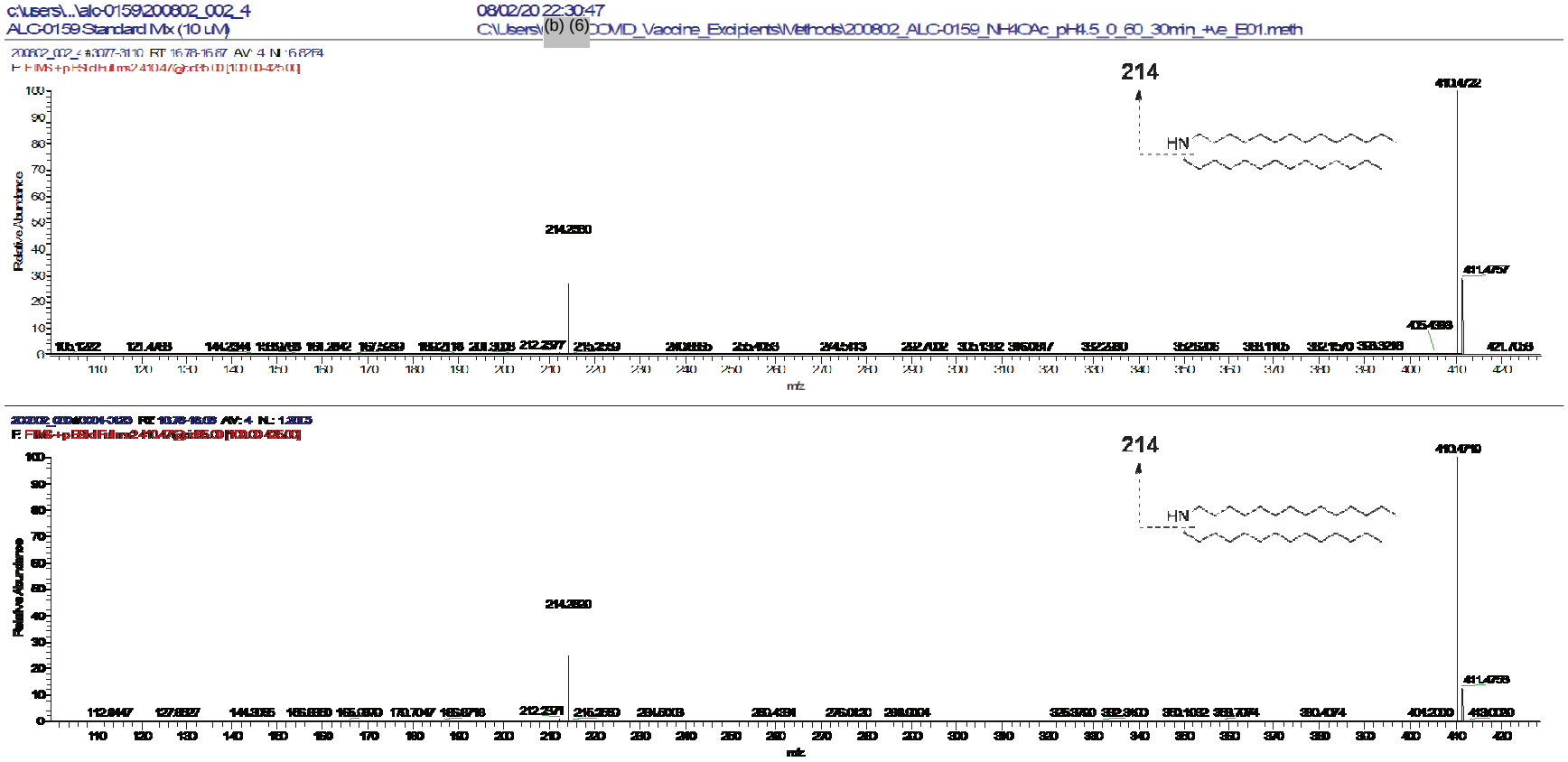


4-ABA – 4-Aminobutyric acid
6-AHA – 6-Aminohexanoic acid

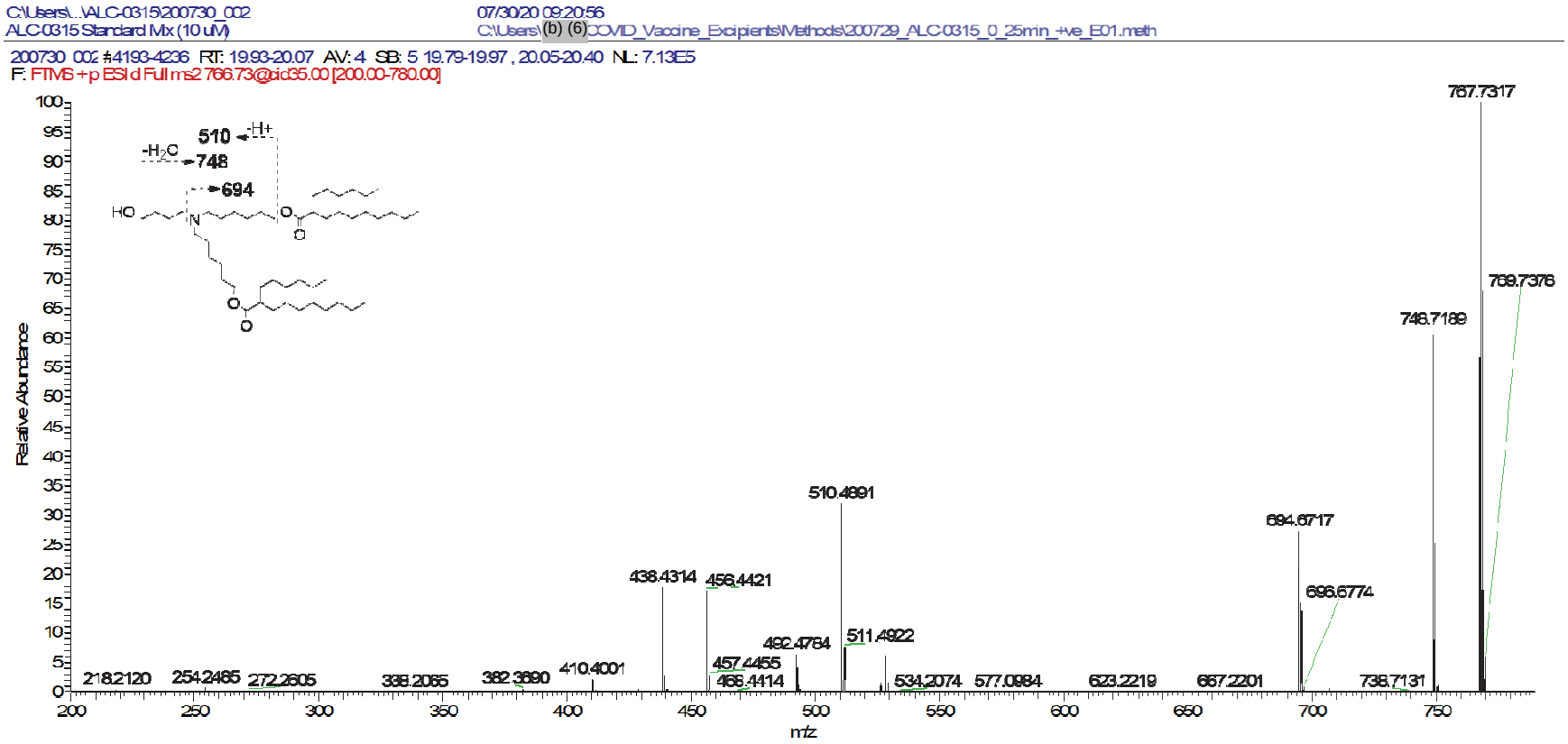
9.9. Mass Spectra for ALC-0159



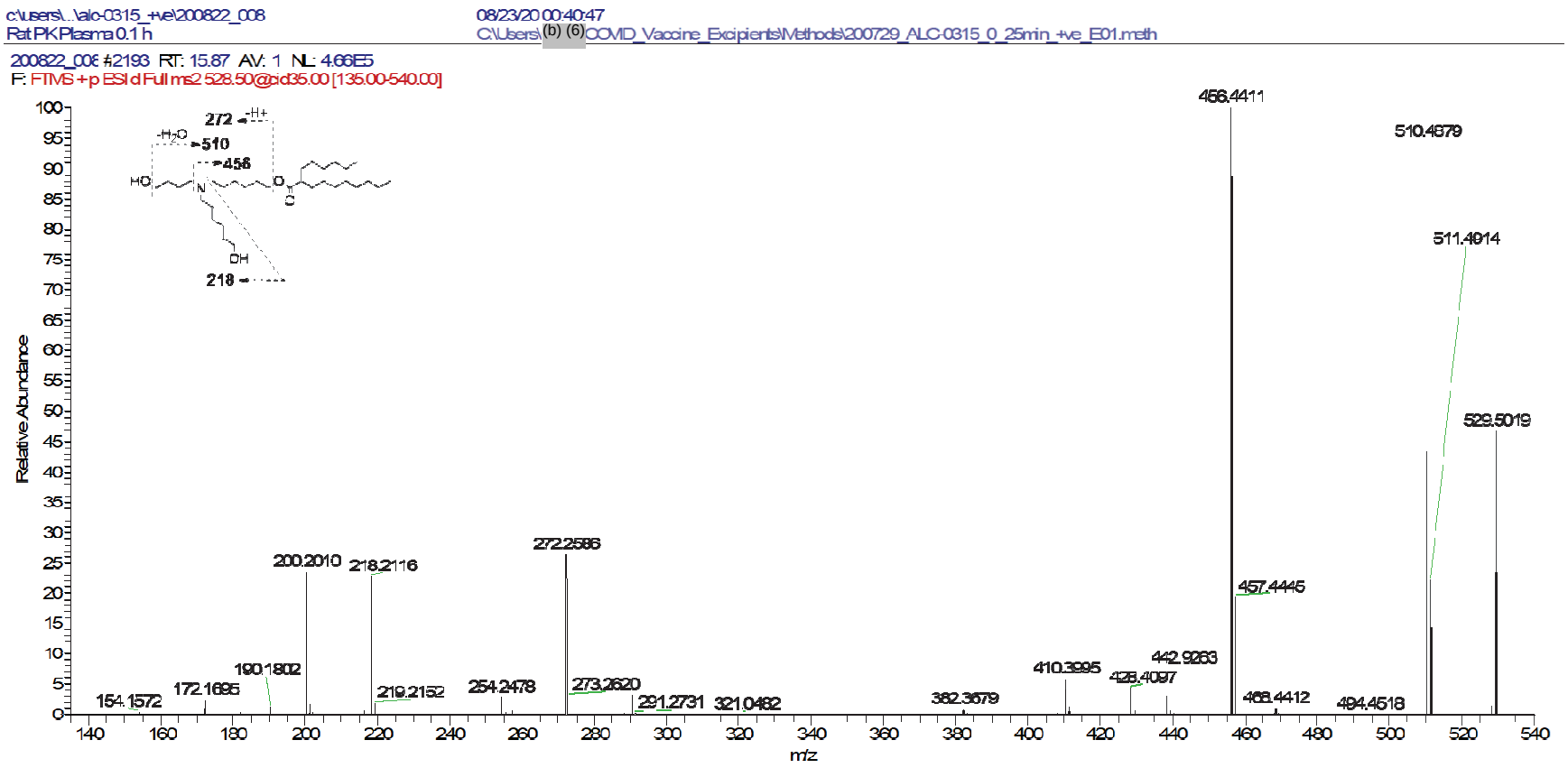
9.10. Mass Spectrum for *N,N*-Ditetradecylamine Reference Standard MS^2 (top) and ALC-0159 *m/z* 410 Metabolite MS^2 (bottom) from Incubation of ALC-0159 with Mouse Hepatocytes



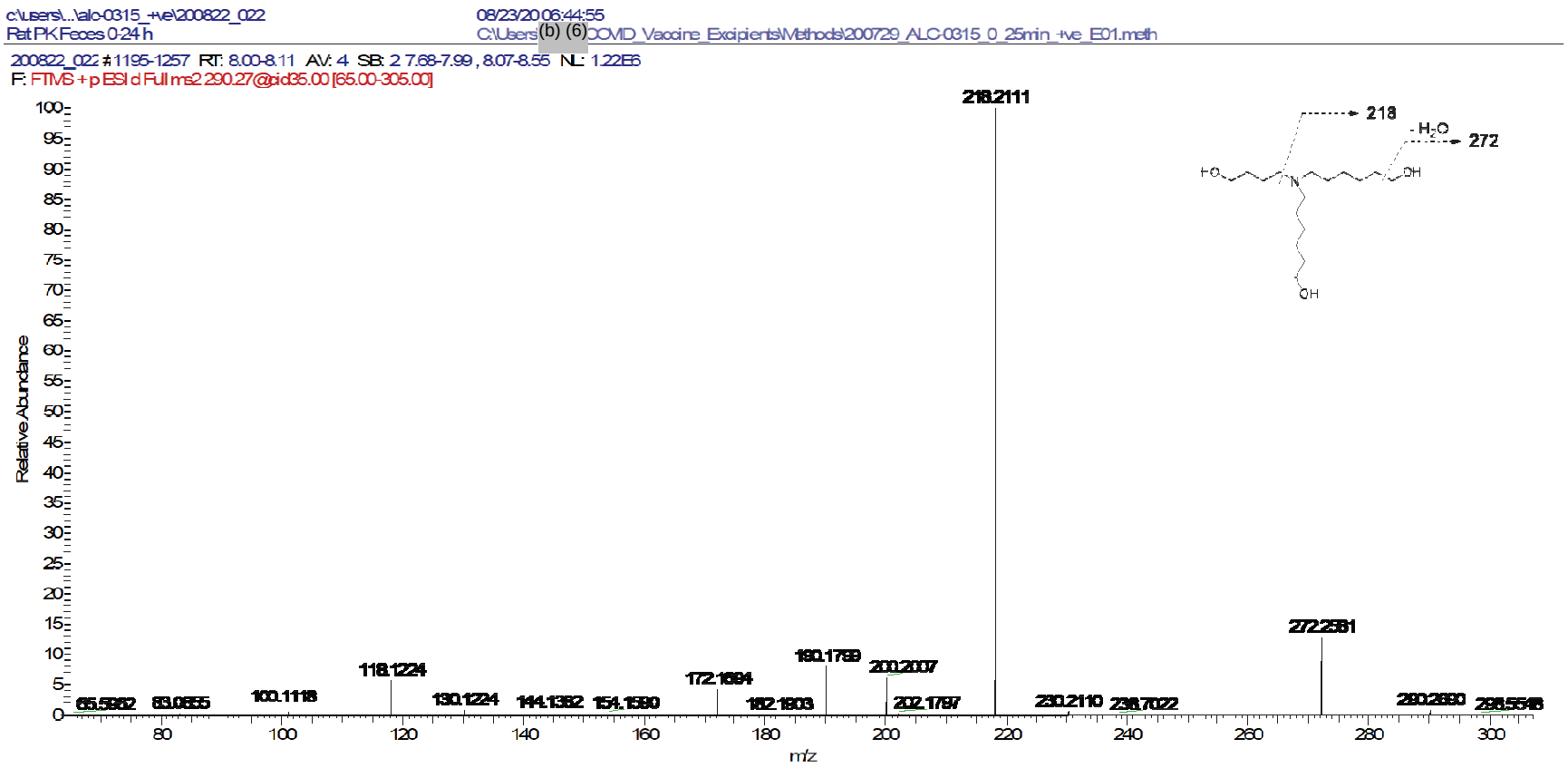
9.11. Mass Spectrum for ALC-0315 *m/z* 766 MS^2



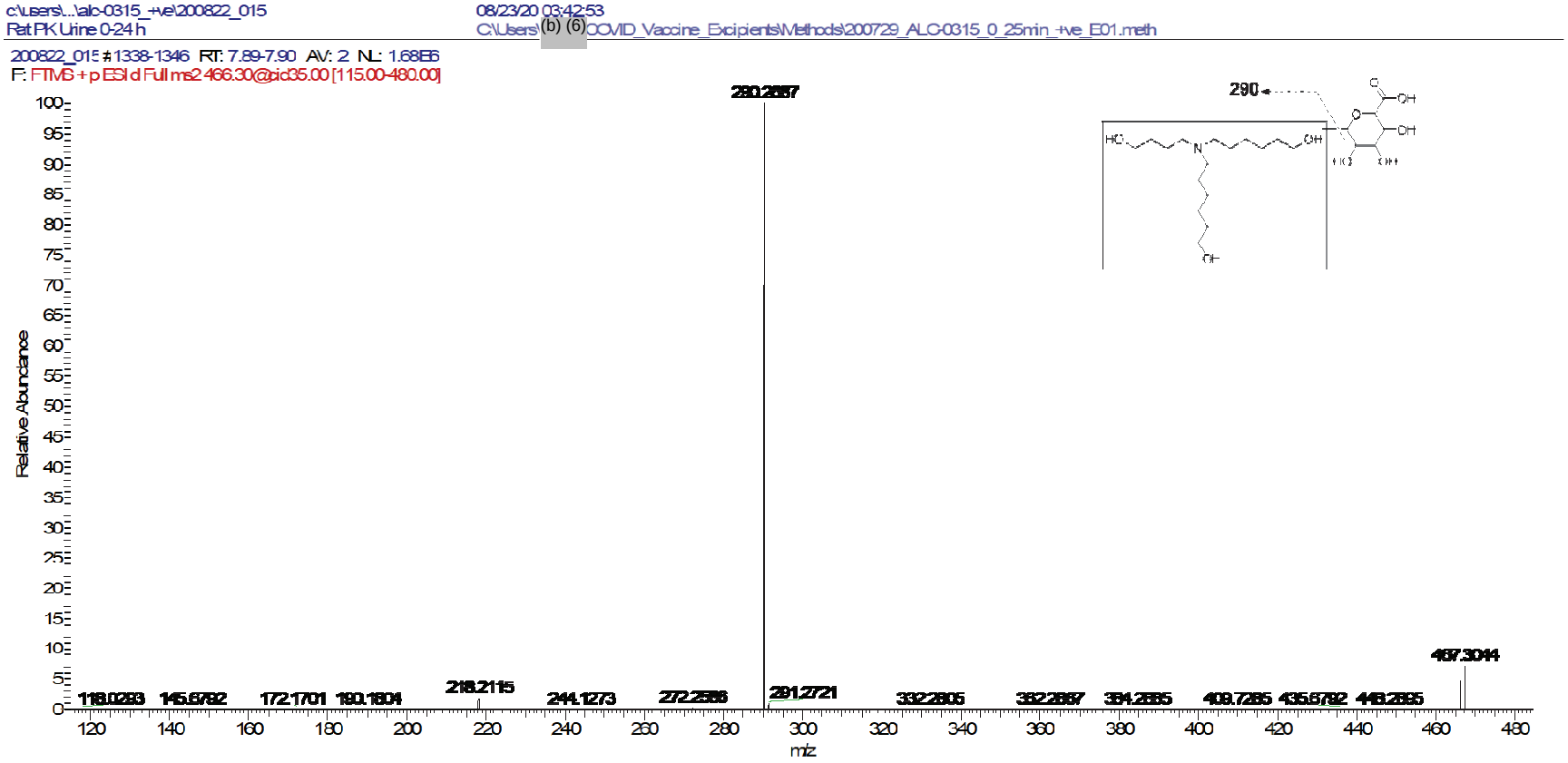
9.12. Mass Spectrum for ALC-0315 *m/z* 528 Metabolite MS^2 in Plasma from a Rat Pharmacokinetics Study



9.13. Mass Spectrum for ALC-0315 *m/z* 290 Metabolite MS^2 in Feces from a Rat Pharmacokinetics Study



9.14. Mass Spectrum for ALC-0315 *m/z* 466 Metabolite MS^2 in Urine from a Rat Pharmacokinetics Study



10. CONTRIBUTING SCIENTISTS

The following scientists were involved in the conduct of this study, and are responsible for the scientific content of this research report.

Contributing ADME Scientist (b) (6)

11. APPROVAL

The author and approver are responsible for the accurate representation of the data in this research report.

(b) (6)
Report Author
Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, USA

(b) (6) .
Report Approver
Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, USA

Document Approval Record

Document Name:	PF-07302048_05Aug20_043725_Investigation of the Biotransformation of ALC-0159 and ALC-0315 In Vitro and In Vivo in Rats
Document Title:	PF-07302048_05Aug20_043725_Investigation of the Biotransformation of ALC-0159 and ALC-0315 In Vitro and In Vivo in Rats

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	11-Sep-2020 15:33:13	Manager Approval
	11-Sep-2020 18:13:55	Author Approval

Final Report Amendment 1

17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Testing Facility Study Number: 20GR142

Alternative Test Article Identifier(s):

PF-07302048: Generic number for COVID-19 vaccine program

BNT162b2 (V9): BNT162b2 (Version 9); RBP020.2; PF-07305885

BNT162b3c: BNT162b3; RBP020.8; PF-07315256

TESTING FACILITY:

Pfizer Worldwide Research & Development
Drug Safety Research & Development
Eastern Point Road
Groton, CT 06340 USA

SIGNATURES

The final report has been amended to clarify and correct the data and/or interpretation of the results following issuance on 13 Nov 2020.

Study Director
(b) (6)

Quality Assurance Statement Signature

The signature for the following individual applies only to the Groton, CT Quality Assurance Statement contained in this study report.

(b) (6)
Regulatory Quality Assurance-Good Laboratory Practices, Pfizer, Groton CT.

For signatures see the Document Approval Record located on the last page of this report amendment.

1. AMENDED TEXT

Section: GLP Compliance Statement

Justification for revision(s): Text is being revised based on feedback from regulatory authorities to clarify that manufacturing of the test articles was conducted non-GMP but characterization of the test articles was conducted under GMP conditions, and that serology analysis was conducted under Good Clinical Laboratory Practice (GCLP).

Current:

This study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58) with the exceptions of the analyses of alpha-1 acid glycoprotein (A1AGP) and alpha-2-macroglobulin (A2M), and testing performed on the test articles BNT162b2 (Version 9 [V9]) and BNT162b3c which were under non-GLP and non-GMP conditions, respectively. These parameters were conducted under non-GLP and non-GMP conditions and were performed according to fit-for-purpose methods. These exceptions did not have an impact on the integrity or data interpretation of the study.

Amended To:

This study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58) with the exceptions of the analyses of alpha-1 acid glycoprotein (A1AGP) and alpha-2-macroglobulin (A2M) which were under non-GLP conditions. Manufacturing of the test articles BNT162b2 (Version 9 [V9]) and BNT162b3c was conducted under non-GMP conditions, while characterization of the test articles was conducted under GMP conditions. Serology analysis was performed in accordance with Good Clinical Laboratory Practice (GCLP). All parameters that were conducted under non-GLP and non-GMP conditions were performed according to fit-for-purpose methods. These exceptions did not have an impact on the integrity or data interpretation of the study.

Regulatory Quality Assurance

Quality Assurance Statement

Title: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study: 20GR142

In accordance with Pfizer policies and Regulatory Quality Assurance procedures for Good Laboratory Practice (GLP), the conduct of this study has been inspected and/or audited as follows. The Individual Quality Assurance Statement for study phase(s) conducted at other site(s) are contained within this report.

Phase Inspected	Audit/Inspection Date GMT	Reporting Date GMT
Report Amendment 1: Nonclinical Study	17-Dec-2020 to 17-Dec-2020	17-Dec-2020

In addition Routine Facility and Process audits are conducted in accordance with RQA SOPs and Site Monitoring Plans.

(b) (6)

Pfizer Confidential

Document Approval Record

Document Name:	Report Amendment
Document Title:	Study 20GR142 Report Amendment 1

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	17-Dec-2020 21:25:01	Quality Assurance Approval
	17-Dec-2020 21:28:51	Author Approval

17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Testing Facility Study Number: 20GR142

Alternative Test Article Identifier(s):

PF-07302048: Generic number for COVID-19 vaccine program

BNT162b2 (V9): BNT162b2 (Version 9); RBP020.2; PF-07305885

BNT162b3c: BNT162b3; RBP020.8; PF-07315256

TESTING FACILITY:
Pfizer Worldwide Research & Development
Drug Safety Research & Development
Eastern Point Road
Groton, CT 06340 USA

SIGNATURES

I approve the report and confirm that the study was conducted in compliance with GLP regulations with the exceptions noted (see GLP Compliance Statement). My interpretation and conclusion of the data accurately reflects the interpretation of the Contributing Scientists and Principal Investigators.

Study Director
(b) (6)

Quality Assurance Statement Signature

The signature for the following individual applies only to the Groton, CT Quality Assurance Statement contained in this study report.

(b) (6)
Regulatory Quality Assurance-Good Laboratory Practices, Pfizer, Groton CT.

For signatures see the Document Approval Record located on the last page of this report.

OTHER STUDY PERSONNEL

The following personnel were involved in the conduct of this study:

Comparative Medicine Activities:	(b) (6)
Ophthalmology Examinations:	
Study Technician(s):	
Study Scientist:	
Study Toxicologist:	
Test Formulations Coordinator: Formulator:	
Clinical Pathology Coordinator:	
Necropsy/Histology Coordinator:	
Biostatistician:	
Safety Biomarkers and Translational Sciences Scientist: Principal Investigators: Serum Antibody Sample Analysis:	
Clinical Pathologist:	
Anatomic Pathologist:	
Peer Review Pathologist	

GLP COMPLIANCE STATEMENT

This study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58) with the exceptions of the analyses of alpha-1 acid glycoprotein (A1AGP) and alpha-2-macroglobulin (A2M), and testing performed on the test articles BNT162b2 (Version 9 [V9]) and BNT162b3c which were under non-GLP and non-GMP conditions, respectively. These parameters were conducted under non-GLP and non-GMP conditions and were performed according to fit-for-purpose methods. These exceptions did not have an impact on the integrity or data interpretation of the study.

ANIMAL WELFARE COMPLIANCE

This study was conducted in accordance with the current guidelines for animal welfare (National Research Council Guide for the Care and Use of Laboratory Animals, 2011). The procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee.

TABLE OF CONTENTS

ABSTRACT

BNT162b2 (Version 9 [V9]) and BNT162b3c are candidate COVID-19 vaccines, which are based on a lipid nanoparticle (LNP)-RNA platform and express the SARS-CoV-2 spike protein or its derivatives. 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 once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats. The reversibility of effects was evaluated following a 3-week recovery phase.

IM administration of BNT162b2 (V9) and BNT162b3c at 30 µg RNA/dose once weekly for a total of 3 doses to Wistar Han rats was tolerated without evidence of systemic toxicity and produced nonadverse inflammatory changes consistent with expected immune responses to vaccines.

At the conclusion of the dosing phase, test article-related immune responses to both vaccines were evident as transient edema and erythema at the injection site after each dose, transient higher mean body temperatures compared with controls after each dose, higher white blood cell count (primarily involving neutrophils, monocytes and large unstained cells), and changes in acute phase reactants (higher [alpha-1 acid glycoprotein and alpha-2-macroglobulin and fibrinogen] and lower [lower albumin and albumin:globulin (AG) ratios] acute phase proteins. These test article-related changes were fully reversed after the recovery phase, with the exception of higher red cell distribution width, higher globulins, and lower AG ratio.

Changes secondary to inflammation included lower mean body weight, lower mean food consumption, transiently lower reticulocyte counts, and minor lower red cell mass at the conclusion of the dosing phase. These changes fully resolved in the recovery phase.

At the conclusion of the dosing phase, nonadverse test article-related microscopic findings consistent with immune activation and an inflammatory response included mixed cell inflammation and edema of the injection sites (which correlated with macroscopic observations of abnormal color, dark/pale and abnormal consistency, firm), increased cellularity of plasma cells and germinal centers of the draining and inguinal lymph nodes (which correlated with macroscopic observation of abnormal size, enlarged), increased cellularity of hematopoietic cells and germinal centers of the spleen (which correlated with macroscopic observation of abnormal size, enlarged and increased spleen weights), and increased cellularity of hematopoietic cells in the bone marrow were noted. These test article-related changes fully recovered, except for partial recovery of enlarged draining and inguinal lymph nodes and microscopic findings of inflammation at the injection sites, increased cellularity of plasma cells and germinal centers in the draining and inguinal lymph nodes and increased cellularity of the germinal centers in the spleen.

In addition, test article-related vacuolation of the periportal hepatocytes in the liver was observed, in the absence of biochemical evidence of liver injury, and may be related to hepatic clearance of PEGylated lipids that are part of the LNP formulation. At the end of the 3-week recovery phase, this finding was completely recovered.

Administration of 3 once weekly doses of BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses in both males and females at the end of the dosing and recovery phases of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals.

In conclusion, BNT162b2 (V9) and BNT162b3c administered via intramuscular injection once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats was tolerated without evidence of systemic toxicity, generated a SARS-CoV-2 neutralizing antibody response, and produced nonadverse changes consistent with an immune or inflammatory response at the conclusion of the dosing phase. At the end of the 3-week recovery phase, full or partial recovery of all findings was observed. Other nonadverse findings included vacuolation in the liver which may be related to hepatic clearance of PEGylated lipids and was noted at the conclusion of the dosing phase and completely recovered. The findings in this study are consistent with those typically associated with the intramuscular administration of LNP-encapsulated mRNA vaccines. Animals administered BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses at the end of the dosing and recovery phases of the study.

1. INTRODUCTION AND OBJECTIVE

BNT162b2 (Version 9 [V9]) and BNT162b3c are candidate COVID-19 vaccines, which consist of an LNP-encapsulated RNA encoding the SARS-CoV-2 spike protein or its derivatives. The objective of this study was to determine the toxicity and development of a specific immune response to the antigens in each of the vaccine candidates following administration of intramuscular (IM) doses once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats. The reversibility of effects was evaluated following a 3-week recovery phase.

2. STUDY RATIONALE

BNT162b2 (V9) and BNT162b3c were evaluated at the highest intended dose (doses up to 30 µg of RNA administered twice) in clinical trials. Therefore, 3 IM administrations of each vaccine at 30 µg RNA for a total of 3 doses were evaluated in the current study in rats on a more accelerated schedule (once weekly) compared to the clinic. The IM route is the clinical route of administration. The rat is a standard rodent test species for use in toxicity studies and has been shown to generate an immune response to very similar types of RNA-based vaccines.

3. MULTI-SITE INFORMATION

Microscopic examination was conducted at Pfizer, Pearl River. Evaluation of Clinical Laboratory parameters was conducted at Pfizer, Pearl River. The analysis for detection of neutralizing antibody titers (serology) to wild type live SARS-CoV-2 virus was conducted at VisMederi, Srl (Siena, Italy).

3.1. Communication Method

The Principal Investigator was responsible for informing the Study Director of any deviations to the protocol or Standard Operating Procedures (SOPs) and unexpected events as they occurred during the respective study phase. Other issues were communicated at the end of the respective study phase prior to the issuance of the report.

3.2. Reporting Method

Clinical Pathology and Anatomic Pathology Principal Investigator's reports are appended to the final study report. Data and interpretation are integrated into the final study report. The Serology Principal Investigator's report is integrated and appended to the final study report.

Methods for each phase are described in the SOP of the respective test site.

4. CONTACT INFORMATION

Pfizer Lead Quality Assurance (QA)[a]	(b) (6) Pfizer Regulatory Quality Assurance-Good Laboratory Practices (RQA-GLP) Eastern Point Road, (b) (6) Groton, CT 06340 (b) (6)
Pfizer Test Site QA[a]	(b) (6) Pfizer Regulatory Quality Assurance-Good Laboratory Practices (RQA-GLP) (b) (6) 401 N. Middletown Road Pearl River, NY 10965 (b) (6)
CRO Test Site[b]	(b) (6)

CRO = Contract Research Or

a. The Pfizer lead and test site QA monitored applicable study phases, audited the final study or Principal Investigator (PI) report(s), and issued QA statement(s) for work conducted at their respective test sites according to RQA-GLP SOPs. Lead QA was responsible for coordination to ensure appropriate overall study monitoring.

b. The CRO test site QA monitored the phase, audited the CRO Principal Investigator's report, and issued a QA Statement according to CRO test site QA SOPs.

5. MATERIALS AND METHODS

For phases of the study conducted at Pfizer Worldwide Research & Development (Pfizer WRD), Groton, CT, details of methods described below are included in the Standard Operating Procedures of Pfizer WRD, Groton, CT and in the SOPs of the respective Pfizer WRD facility conducting those activities.

Minor deviations from the protocol and/or current standard operating procedures occurred and did not affect the quality, integrity or interpretations of the data or the conclusions of the study. The deviations are documented in the study records and are discussed in the appropriate section of the report.

5.1. Study Schedule

Study Initiation Date (date protocol signed):	23 Jun 2020
Experimental Start Date (first day of study-specific data collection):	24 Jun 2020
First Day of Dosing (Day 1):	06 Jul 2020
First Day of Recovery Phase:	23 Jul 2020
Dosing Phase Necropsy (first 10 animals/sex/group):	22 Jul 2020
Recovery Phase Necropsy (remaining animals):	13 Aug 2020
Experimental Completion Date (last day of study-specific data collection):	13 Aug 2020

5.2. Test and Control Articles

5.2.1. Test Articles

5.2.1.1. BNT162b2 (V9)

Test Article Number:	BNT162b2 (V9)
Lot Number:	COVVAC/270320
Manufacturer:	Polymun
Composition	0.5 mg/mL RNA encoding the full SARS-CoV-2 Spike (S) P2 variant protein
Expiration Date:	27 Sep 2020
Storage Conditions:	Frozen at -80°C, protected from light
Composition:	See Certificate of Analysis in Appendix C.

5.2.1.2. BNT162b3c

Test Article Number:	BNT162b3c
Lot Number:	BCV/040620
Manufacturer:	Polymun
Composition	0.5 mg/mL RNA encoding Membrane-anchored, trimerized variant of the RBD of the SARS-CoV-2 S protein
Expiration Date:	04 Dec 2020
Storage Conditions:	Frozen at -80°C, protected from light
Composition:	See Certificate of Analysis in Appendix C.

5.2.2. Control Article(s)

5.2.2.1. Vehicle

A solution of 0.9% sterile saline was used to dose the control animals (Group 1).

Excipient:	0.9% sterile saline
Lot Number:	J8L247
Expiration Date:	31 Mar 2021

5.2.3. Test Article Formulation and Analyses

Test Article Numbers: BNT162b2 (V9) and BNT162b3c

Type of Formulation:	Suspension
Method of Preparation:	Thawing of frozen formulation
Frequency of Preparation:	06 Jul 2020, 13 Jul 2020, and 20 Jul 2020
Storage:	Room temperature, protected from light
Formulation Handling at Time of Dispensing for Dosing:	Formulations were gently inverted to mix to ensure uniformity prior to dose administration
Stability:	2 hours from the time thaw was completed[a]
Concentration Analyses:	Not applicable; material was utilized as supplied

a. Reference: DOSAGE AND ADMINISTRATION INSTRUCTIONS FOR BNT162 (PF-07302048) VACCINE, 0.5 MG/ML (C459-INX100407124-V4.0). NOTE: Although the information in this reference document is not specific to the test articles utilized in this study, it was for the same platform of vaccines and was deemed appropriate for use.

5.3. Test System

Species:	Rat
Strain/Breed/Origin:	Wistar Han (Crl:WI[Han])
Animal Use Protocol (AUP) Number:	GTN-2011-00314
Source:	Charles River Laboratories Raleigh, NC
Age at Dose Initiation:	9 weeks
Weight at Dose Initiation:	Males: 243.1 grams - 291.6 grams Females: 172.9 grams - 209.5 grams

5.3.1. Acclimation

Animals were acclimated to the laboratory environment for a minimum of 13 days prior to initiation of dosing.

5.3.2. Identification

Animals were identified by a radio frequency identification device (RFID) implanted by the vendor (subscapular region) that was associated with a unique identification number for each animal. Each cage was labeled with a cage card for each animal in the cage.

5.3.3. Allocation and Randomization

Clinically acceptable animals were allocated to study groups following the review of data collected prior to the initiation of dosing and using a computer-assisted randomization procedure based on body weights.

5.4. Housing and Environmental Conditions

Caging:	Housed individually in suspended cages
Bedding:	Enrich-n'Pure®, The Andersons, Inc.
Temperature:	68°F-79°F
Humidity:	30%-70%
Lighting:	Approximate 12-hour light, 12-hour dark cycle.
Water:	Municipal drinking water, further purified by reverse osmosis, was provided ad libitum.
Diet:	Certified Irradiated Rodent Diet 5002 (PMI Feeds Inc.) was provided ad libitum. Lot number(s) are included in the raw data.

There are no known contaminants in the food or water that interfered with the quality or integrity of the data.

5.5. Experimental Groups

Group Number	Test Article or Vehicle Dose (µg RNA/Dose Day)	Dose Volume (µL/injection site)[a]	Animal Numbers	
			Males	Females
1	0[b]	60	1-15	46-60
2	30[c]	60	16-30	61-75
3	30[d]	60	31-45	76-90

a. Each animal received a single intramuscular injection on each dose day.
b. Sterile saline.
c. BNT162b2 (V9).
d. BNT162b3c.

Doses were administered by a single intramuscular injection (60 µL) on each dosing day (Days 1, 8, and 15) into the left hindlimb quadriceps muscle.

The first 10 animals/sex/group, by ascending animal order, were designated for necropsy at the end of the dosing phase. The remaining 5 animals were retained for the recovery phase.

5.6. Observations and Measurements

5.6.1. Clinical Observations/Measurements

General (Cageside) Clinical Observations:	**Days of Study**	**Time Points**
	Prior to the Initiation of Dosing (PID)	Once daily
	Nondosing Days (Dosing Phase)	Twice daily, except on days when detailed clinical observations were performed, then only once daily
	Dosing Days (Dosing Phase)	Predose, except on days that predose detailed clinical observations were performed, 4 hours after the last animal was dosed, and at the end of the workday. On 06 Jul 2020 (Day 1), clinical signs were not conducted at the end of the workday for Animals 001-090.
	Recovery Phase Days	Twice daily
Detailed Clinical Observations:	Detailed clinical observations were performed twice prior to the initiation of dosing, twice weekly at approximately the same time body weights were performed, and on the day(s) of necropsy.	
Body Weight:	All animals were weighed twice prior to the initiation of dosing on PID Phase Days 1 and 6, predose on Dosing Phase Days 1, 8, and 15; on Dosing Phase Days 4 and 11 (nondosing), and a fasted weight was collected just prior to scheduled necropsy. Body weights were collected on Recovery Phase Days 1, 4, 8, 11, 15, 18, and 21.	
Food Consumption:	Quantitative food consumption was recorded on Dosing Phase Days 4, 8, 11, and 15 and on Recovery Phase Days 4, 8, 11, 15, 18, and 21.	
Ophthalmology:	Ophthalmic examinations were performed once prior to the initiation of dosing (following randomization) on PID Phase Days 7/8 (males/females) and on Dosing Phase Days 15/16 (males/females). Recovery animals were not examined at the end of the recovery phase. See the Ophthalmology Report in Appendix B for complete materials and methods.	
Injection Site Scoring (Dermal Assessment):	Injection sites were observed during the dosing phase once predose and approximately 4 and 24 hours postdose on all animals. Animals with a score of 2 or greater at 24 hours postdose had additional evaluations at 48 and 72 hours postdose. Animals with a continued score of 2 or greater at 72 hours postdose had additional evaluations at 120 and 144 hours postdose. After dosing on Day 15, a 72-hour postdose evaluation was conducted on recovery animals only. Injection site score was recorded according to a standardized rating scale (Draize, 1959). On Dosing Phase Day 1 (06 Jul 2020), predose dermal assessments were collected on all animals for right-side injection sites (noninjection site), and at 4 hours postdose, dermal assessments were collected on Animals 1-7, 9 (Group 1, Males), and 46-58 (Group 1, Females) for right-side injection sites (noninjection site).	
Body Temperature:	Body temperature was collected on all animals once prior to the initiation of dosing on PID Phase Day 6, predose on Dosing Phase Days 1, 8, and 15, and at approximately 4 and 24 hours postdose from all animals.	

5.6.2. Clinical Laboratory Measurements

Schedule for Collection of Samples for Clinical Laboratory Measurements			
Parameter	**Day of Study**		
	Dosing Phase		**Recovery Phase**
	Day 4	Day 17[e]	Day 22
Hematology	X[a,c]	X[c]	X[c]
Coagulation	NA	X[c]	X[c]
Clinical Chemistry (Core Chemistry)	X[b,c]	X[c]	X[c]
Clinical Chemistry (Other Biomarkers – Acute Phase Proteins)/Serum[d]	X[b,c]	X[c]	X[c]
Urinalysis	NA	X	X

NA = Not applicable; X = Scheduled collection.
a. First 7 animals/sex/group.
b. Last 8 animals/sex/group.
c. Blood samples were collected from animals in a fasted state, with the exception of same day redraws.
d. Assay performed using shared clinical chemistry sample.
e. Evaluated on animals scheduled for necropsy.

See the Clinical Pathology Report in Appendix B for complete materials and methods.

5.6.3. Antibody (Serology) Response to Vaccine Components

Sample Collection and Storage Conditions	
Groups:	1-3
Collection Intervals:	PID Phase Day 8 and Dosing Phase Day 17[a], and Recovery Phase Day 21[a]
Collection Time Points:	PID Phase Day 8, Dosing Phase Day 17, and Recovery Phase Day 21: Once
Animals/Time Point:	All animals
Anticoagulant:	No anticoagulant
Collection Volume per Sample:	PID Phase Day 8: Approximately 0.7 mL Dosing Phase Day 17 and Recovery Phase Day 21: Approximately 1 mL
Sample Processing:	Samples were processed and stored as appropriate within 2 hours of collection
Sample Storage Conditions:	Approximately -60°C or lower

PID = Prior to initiation of dosing.
a. Samples collected prior to necropsy.

All samples collected were sent in one shipment after completion of the last blood sample collection.

Antibody Analysis	
Analysis of Samples from Control Animals (Group 1):	All samples were analyzed
Analysis of Samples from Animals Administered Test Article:	All samples were analyzed for a neutralizing antibody response to the antigens in BNT162b2 (V9) and BNT162b3c

Incurred Sample Reanalysis (ISR)/Project Numbers	
Antibody (Serology) Sample Analysis was conducted under the following Qualified Method ID:	PFZ_20GR142-WO4_MN SarsCov2_V2_20200924_GL

See the Serology Report in Appendix B for complete materials and methods.

5.7. Postmortem Observations

Animals (10/sex/group) were euthanized on Dosing Phase Day 17 (2 days after the last dose). Remaining animals were euthanized on Recovery Phase Day 22, the last day of the Recovery Phase (surviving animals).

Necropsy, tissue collection, organ weights, macroscopic tissue evaluation, and microscopic examination were performed.

Bone marrow smears were collected from all animals.

See the Anatomic Pathology Report in Appendix B for complete materials and methods.

5.8. Statistical Analysis

Statistical analyses of body weight, body weight change, and food consumption data were conducted in Pristima and analyses of body temperature and injection site scores were conducted by DSRD Statistics using iStats v1.0 with the methods outlined below. All analyses were performed separately for each sex.

Descriptive statistics were generated for each parameter and group at each scheduled sampling time or each time interval. Statistical tests were conducted at the 5% and 1% significance levels.

Analyses of body weight and food consumption parameters were done on measurements collected for each animal at the scheduled sampling times or time intervals. In addition, body weight change at selected intervals was analyzed. Analysis of body temperature was based on the maximum body temperature after injection for each animal. Analysis of injection site score was based on the average irritation score after injection for each animal.

A nonparametric (rank-transform) one way analysis of variance (ANOVA) on all groups was conducted, with two-sided pairwise comparisons of Groups 2 and 3 to Group 1 using Dunnett's test. Average ranks were assigned to ties.

For statistical analysis performed for contributing scientist activities/measurements, see the corresponding report in Appendix B.

5.9. Data Acquisition

The following primary computer applications were used for the collection of data.

Computer Application	Data Collected/Usage
Pristima Preclinical Data Management Suite (Version 7.4.3)	In-life activities
DVMAX Research Version 3.1.2	Animal health records
Microsoft Excel	Sample tracking and antibody immunoassay result storage. Duplicate titration for each sample, provided two neutralization titers (MNt) for each sample. Information was documented according to VisMederi, Srl Standard Operating Procedures (WI-MNSARS-CoV-2) are stored in an Excel sheet (the basic format is provided in dedicated VisMederi, Srl procedure).
iStats Version 1.0	Statistical analysis

For data acquisition systems and version numbers of each of these systems used for contributing scientist/principal investigator activities/measurements, see the corresponding report in Appendix B.

5.10. Data Management and Archives

Data	Location of Archive
Raw data, documentation, protocol and amendments, final report, and any specimens generated at the Test Facility	Pfizer, Groton, CT
Raw data and documents electronically archived	Pfizer OpenLab archive system or locked and retained in the source computerized system, as defined as per SOP.
Materials are retained in accordance with the Enterprise Records Retention Schedule.	
Raw data, working sheets and any template required by method procedure are archived as hard copies (original documents) in fireproof archives up to 25 years. Electronic format outputs are regularly backed up and archived in Microsoft cloud.	

6. RESULTS

6.1. Clinical Observations/Measurements

6.1.1. Mortality

Individual animal mortality data are included in Appendix 1.

There was no unscheduled euthanasia. All animals administered BNT162b2 (V9) or BNT162b3c survived to scheduled necropsy at the end of the dosing or recovery phase of the study.

6.1.2. Clinical Signs

An incidence summary of clinical signs is presented in Table 1. Individual animal clinical signs are included in Appendix 2.

There were no test article-related clinical signs noted for animals administered BNT162b2 (V9) or BNT162b3c during the dosing or recovery phase.

6.1.3. Body Weight

Group mean body weight data are presented in Table 3. Group mean body weight change during interval data are presented in Table 4. Individual animal body weight data are included in Appendix 4. Individual animal body weight change during interval data are included in Appendix 5.

Dosing Phase

No test article-related mean body weight changes were noted for animals administered BNT162b2 (V9) during the dosing phase.

Test article-related lower mean body weight (0.93x-0.94x control) was noted in males only on Days 11 and 15 for BNT162b3c during the dosing phase.

Recovery Phase

Test article-related higher mean body weight (1.05-1.06x control) was noted in males only on Recovery Days 11, 15, 18 and 21 for animals administered BNT162b2 (V9).

No test article related body weight changes were noted for animals administered BNT162b3c during the recovery phase.

Other differences between test article and control group were not test article-related due to the small magnitude of the change, inconsistent direction of the difference, and/or inconsistency of the response.

6.1.4. Food Consumption

Group mean food consumption data are presented in Table 5. Individual animal food consumption data are included in Appendix 6.

Dosing Phase

Test article-related lower mean food consumption (0.83x-0.87x control) was noted on Days 4 and 11 for animals administered BNT162b2 (V9) during the dosing phase.

Test article-related lower mean food consumption (0.76x-0.92x control) was noted on Days 4 and 11 for animals administered BNT162b3c during the dosing phase.

Recovery Phase

Test article-related higher mean food consumption (1.08x-1.35x control) was noted throughout the recovery phase for male animals administered BNT162b2 (V9).

Test article-related higher mean food consumption (1.08x-1.30x control) was noted on Recovery Phase Days 4 and 11 for male animals administered BNT162b3c.

Other differences between test article and control group were not test article-related due to the small magnitude of the change, inconsistent direction of the difference, and/or inconsistency of the response.

6.1.5. Dermal Assessment

Group mean dermal assessment data are included in Table 12. Individual dermal assessment data are included in Appendix 12.

Dosing Phase

BNT162b2 (V9)-related injection site edema Grade 2 (slight, edges of area well defined by definite raising) or Grade 3 (moderate, raised approximately 1 mm) were noted in all animals (except Animal 17), and occurred following dosing on Days 1, 8 and/or 15 (see Text Table 1). The edema was generally observed up to 72 hours postdose, and fully resolved prior to dose administration on Days 8 and 15. Erythema was also observed at the injection site in all animals (except Animals 16-21 and 30), following each dose administration, however, it was only a Grade 1 (very slight, barely perceptible) and fully resolved prior to the next dose administration.

BNT162b3c-related injection site edema Grade 2 (slight, edges of area well defined by definite raising) or Grade 3 (moderate, raised approximately 1 mm) were noted in all animals, and occurred following dosing on Days 1, 8 and/or 15 (see Text Table 2). The edema was generally observed up to 72 hours postdose, and fully resolved prior to dose administration on Days 8 and 15. Erythema was also observed at the injection site in all animals (except Animal 39), following each dose administration, however, it was only a Grade 1 (very slight, barely perceptible) and fully resolved prior to the next dose administration.

Text Table 1. BNT162b2 (V9) Animals with Injection Site Edema Score ≥ 2

Animal	Clinical Sign	Total Number of Days (Dosing Phase Study Day of Occurrence)
16 M	Edema, Grade 2	1 (D16: 24 HPD)
18 M	Edema, Grade 2	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
19 M	Edema, Grade 2	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
20 M	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
21 M	Edema, Grade 2	6 (D2: 24 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
22 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
23 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
24 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
25 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)

Text Table 1. BNT162b2 (V9) Animals with Injection Site Edema Score ≥ 2 - Cont'd

Animal	Clinical Sign	Total Number of Days (Dosing Phase Study Day of Occurrence)
26 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
27 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
	Edema, Grade 3	2 (D16: 24 HPD; D17: 48 HPD)
28 M	Edema, Grade 2	3 (D2: 24 HPD; D3: 48 HPD; D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
29 M	Edema, Grade 2	1 (D11: 72 HPD)
	Edema, Grade 3	2 (D 9: 24 HPD; D10: 48 HPD)
30 M	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
61 F	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
62 F	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD)
	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
63 F	Edema, Grade 2	8 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
	Edema, Grade 3	2 (D16: 24 HPD; D17: 48 HPD)
64 F	Edema, Grade 2	9 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D17: 48 HPD)
	Edema, Grade 3	1 (D16: 24 HPD)
65 F	Edema, Grade 2	2 (D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
66 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
67 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120, D7: 144; D17: 48 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD)
68 F	Edema, Grade 2	2 (D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
69 F	Edema, Grade 2	8 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D17: 48 HPD)
	Edema, Grade 3	1 (D16: 24 HPD)
70 F	Edema, Grade 2	2 (D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
71 F	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
72 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
73 F	Edema, Grade 2	10 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
74 F	Edema, Grade 2	7 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D11: 72 HPD; D16: 24 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)

Text Table 1. BNT162b2 (V9) Animals with Injection Site Edema Score ≥ 2 - Cont'd

Animal	Clinical Sign	Total Number of Days (Dosing Phase Study Day of Occurrence)
75 F	Edema, Grade 2	8 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD)
	Edema, Grade 3	2 (D16: 24 HPD; D17: 48 HPD)

Note: Dosing Days = 1, 8, and 15.
D = Dosing Phase Day; F = Female; HPD = Hours postdose M = Male.
Grade 0 = No edema; 1 = Very slight edema (barely perceptible); 2 = Slight edema (edges of area well defined by definite raising); 3 = Moderate edema (raised approximately 1 millimeter); 4 = Severe edema (raised more than 1 millimeter and extends beyond the area of exposure).

Text Table 2. BNT162b3c Animals with Injection Site Edema Score ≥ 2

Animal	Clinical Sign	Total Number of Days (Dosing Phase Study Day of Occurrence)
31 M	Edema, Grade 2	4 (D2: 24 HPD; D3: 48 HPD; D11: 72 HPD; D16: 24 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D17: 48 HPD)
32 M	Edema, Grade 2	2 (D9: 24 HPD; D10: 48 HPD)
33 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD: D10: 48 HPD)
34 M	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D9: 24 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	1 (D10: 48 HPD)
35 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
36 M	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
37 M	Edema, Grade 2	7 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
38 M	Edema, Grade 2	3 (D11; 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10:48 HPD)
39 M	Edema, Grade 2	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
40 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D 9: 24 HPD; D10: 48 HPD)
41 M	Edema, Grade 2	1 (D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
42 M	Edema, Grade 2	3 (D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD; D10: 48 HPD)
43 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D11:72 HPD)
	Edema, Grade 3	4 (D 9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
44 M	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
45 M	Edema, Grade 2	7 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
	Edema, Grade 3	2 (D9: 24 HPD: D10: 48 HPD)
76 F	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
77 F	Edema, Grade 2	1 (D13: 120 HPD)

Text Table 2. BNT162b3c Animals with Injection Site Edema Score ≥ 2 - Cont'd

Animal	Clinical Sign	Total Number of Days (Dosing Phase Study Day of Occurrence)
	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
78 F	Edema, Grade 2	1 (D13: 120 HPD)
	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
79 F	Edema, Grade 2	7 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D11: 72 HPD; D17: 48 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD;)
80 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D11: 72 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
81 F	Edema, Grade 2	2 (D9: 24 HPD; D11: 72 HPD)
	Edema, Grade 3	3 (D10: 48 HPD; D16: 24 HPD; D17: 48 HPD)
82 F	Edema, Grade 2	2 (D11: 72 HPD; D17: 48 HPD)
	Edema, Grade 3	3 (D9: 24 HPD; D10: 48 HPD; D16: 24 HPD)
83 F	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD;)
	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
84 F	Edema, Grade 2	9 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D17: 48 HPD)
	Edema, Grade 3	1 (D16: 24 HPD)
85 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D16: 24 HPD)
	Edema, Grade 3	4 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D17: 48 HPD)
86 F	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
87 F	Edema, Grade 2	5 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD;)
	Edema, Grade 3	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
88 F	Edema, Grade 2	6 (D2: 24 HPD; D3: 48 HPD; D4: 72 HPD; D6: 120 HPD; D7: 144 HPD; D9: 24 HPD)
	Edema, Grade 3	4 (D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
89 F	Edema, Grade 2	5 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D16: 24 HPD; D17: 48 HPD)
90 F	Edema, Grade 2	6 (D9: 24 HPD; D10: 48 HPD; D11: 72 HPD; D13: 120 HPD; D16: 24 HPD; D17: 48 HPD)

Note: Doing Days = 1, 8, and 15.
D = Dosing Phase Day; F = Female; HPD = Hours postdose; M = Male.
Grade 0 = No edema; 1 = Very slight edema (barely perceptible); 2 = Slight edema (edges of area well defined by definite raising); 3 = Moderate edema (raised approximately 1 millimeter); 4 = Severe edema (raised more than 1 millimeter and extends beyond the area of exposure).

Recovery Phase

BNT162b2 (V9)-related injection site edema Grade 2 (slight, edges of area well defined by definite raising) or Grade 3 (moderate, raised approximately 1 mm) was noted in 2/5 males and 5/5 females following dosing on Day 15 (see Text Table 3). The edema was generally observed up to 72 hours postdose, and fully resolved. Erythema was also observed at the injection site in 2/5 females after the final dose administration, however, it was only Grade 1 (very slight, barely perceptible) and fully resolved.

BNT162b3c -related injection site edema Grade 2 (slight, edges of area well defined by definite raising) or Grade 3 (moderate, raised approximately 1 mm) was noted in 4/5 males and 5/5 females following dosing on Day 15 (see Text Table 4). The edema was generally observed up to 72 hours postdose, and fully resolved. Erythema was also observed at the injection site in 4/5 females after the final dose administration, however, it was only Grade 1 (very slight, barely perceptible) and fully resolved.

Text Table 3. BNT162b2 (V9) Animals with Injection Site Edema Score ≥ 2

Animal	Clinical Sign	Total Number of Days (Recovery Study Day of Occurrence)
26 M	Edema, Grade 2	1 (RPD1; 72 HPD)
30 M	Edema, Grade 2	1 (RPD1; 72 HPD)
71 F	Edema, Grade 2	1 (RPD1; 72 HPD)
72 F	Edema, Grade 3	1 (RPD1; 72 HPD)
73 F	Edema, Grade 2	1 (RPD1; 72 HPD)
74 F	Edema, Grade 2	1 (RPD1; 72 HPD)
75 F	Edema, Grade 3	1 (RPD1; 72 HPD)

F = Female; HPD = Hours post dose; M = Male; RPD = Recovery Phase Day
Grade 0 = No edema; 1 = Very slight edema (barely perceptible); 2 = Slight edema (edges of area well defined by definite raising); 3 = Moderate edema (raised approximately 1 millimeter); 4 = Severe edema (raised more than 1 millimeter and extends beyond the area of exposure).

Text Table 4. BNT162b3c Animals with Injection Site Edema Score ≥ 2

Animal	Clinical Sign	Total Number of Days (Recovery Study Day of Occurrence)
41 M	Edema, Grade 2	1 (RPD1: 72 HPD)
43 M	Edema, Grade 2	1 (RPD1: 72 HPD)
44 M	Edema, Grade 2	1 (RPD1: 72 HPD)
45 M	Edema, Grade 2	1 (RPD1: 72 HPD)
86 F	Edema, Grade 2	1 (RPD1: 72 HPD)
87 F	Edema, Grade 3	1 (RPD1: 72 HPD)
88 F	Edema, Grade 3	1 (RPD1: 72 HPD)
89 F	Edema, Grade 3	1 (RPD1: 72 HPD)
90 F	Edema, Grade 2	1 (RPD1: 72 HPD)

F = Female; HPD = Hours post dose; M = Males; RPD = Recovery Phase Day.
Grade 0 = No edema; 1 = Very slight edema (barely perceptible); 2 = Slight edema (edges of area well defined by definite raising); 3 = Moderate edema (raised approximately 1 millimeter); 4 = Severe edema (raised more than 1 millimeter and extends beyond the area of exposure).

6.1.6. Body Temperature

Group mean body temperature data are included in Table 13. Individual body temperature data are included in Appendix 13.

Test article-related higher mean body temperature differences from control were noted on Days 1 (+0.42°C-0.54°C), 8 (+0.66°C-0.98°C), and 15 (+0.13°C-1.03°C) following dose administration of BNT162b2 (V9).

Test article-related higher mean body temperature differences from control were noted on Days 1 (+0.50°C-0.71°C), 8 (+0.92°C-1.26°C) and 15 (+0.33°C-1.09°C) following dose administration of BNT162b3c.

Additional body temperature evaluations were not needed at 48 and 72 hours postdose as individual animal body temperatures were ≤40°C at 24 hours postdose.

6.1.7. Ophthalmology

The complete Ophthalmology Report is included in Appendix B and a summary of the results is included below.

There were no test article-related ophthalmic findings noted at the conclusion of the dosing phase. Recovery phase examinations were not performed due to no findings observed at the conclusion of the dosing phase.

6.2. Clinical Laboratory Measurements

The complete Clinical Pathology Report is included in Appendix B and a summary of the results is included below.

Dosing Phase

Test article-related hematology and coagulation findings were similar in rats administered either BNT162b2(V9) or BNT162b3c and included higher mean white blood cell (WBC) counts and fibrinogen concentrations, lower (Day 4) and higher (Day 17) reticulocyte counts, and lower red blood cell mass (red blood cell count, hemoglobin and hematocrit) as compared with controls.

Higher WBC primarily involved higher neutrophils, monocytes and large unstained cells , but also eosinophils and basophils. They were present on Days 4 and 17, with higher counts on Day 17 than Day 4. On Day 17, there were also test article-related higher fibrinogen concentrations in both sexes. Hypersegmented neutrophils were present on peripheral blood smears of test article-dosed animals.

In addition, there were test article-related transiently lower reticulocyte counts on Day 4, and higher reticulocytes on Day 17 (females only) with attendant expected changes in RBC indices (higher mean cell hemoglobin concentration; males on Day 4; lower mean cell hemoglobin [MCH] and higher red cell distribution width on Day 17; both sexes). These

were associated with lower RBC mass on Days 4 and 17 (comparable on both days or slightly lower on Day 17).

Test article-related clinical chemistry findings were similar in rats administered either BNT162b2(V9) or BNT162b3c and included higher mean alpha-1 acid glycoprotein and alpha-2-macroglobulin and lower AG ratios (primarily due to lower albumin with slight contribution from higher globulins) on Days 4 and 17 in both sexes.

Recovery Phase

All test article-related hematology and coagulation changes noted in the dosing phase were fully reversed after a 3-week recovery phase, with the exception of higher red cell distribution width.

All test article-related clinical chemistry changes noted in the dosing phase were fully reversed after a 3-week recovery phase, with the exception of higher globulins in males administered BNT162b2(V9) and females administered BNT162b2(V9) and BNT162b3c and lower AG ratio in females administered BNT162b2(V9).

There were no test article-related findings noted in urinalysis parameters in the dosing or recovery phase.

6.2.1. Bone Marrow Assessment

The complete Clinical Pathology Report is included in Appendix B and a summary of the results is included below.

Bone marrow smears were prepared for all animals and were not examined.

6.3. Antibody (Serology) Analysis

The complete Serology Report is included in Appendix B and a summary of the results is included below.

Administration of 3 once weekly doses of BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses in males and females at the end of the dosing (Day 17) and recovery phases (Day 21) of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals.

6.4. Postmortem Observations

The complete Anatomic Pathology Report is included in Appendix B and a summary of the results is included below.

Dosing Phase

Test article-related organ weight differences included higher absolute and relative (to body and brain weight) spleen weights in males and females administered BNT162b2 (V9) or BNT162b3c.

Test article-related macroscopic findings included large draining lymph nodes (abnormal size, enlarged) and dark/pale and/or firm injection sites (abnormal color, dark/pale and/or abnormal consistency, firm) in animals administered BNT162b2 (V9) or BNT162b3c, and large spleen and inguinal lymph nodes (abnormal size, enlarged) in animals administered BNT162b3c.

Organs with test article-related microscopic findings included the injection site (mixed cell inflammation and edema), draining and inguinal lymph nodes (increased cellularity, plasma cells and germinal centers), liver (hepatocellular vacuolation), spleen (increased cellularity, hematopoietic cells and germinal centers), and bone marrow (increased cellularity, hematopoietic cells) in both males and females administered BNT162b2 (V9) or BNT162b3c.

Recovery Phase

No test article-related organ weight changes were noted at the end of the recovery phase.

Test article-related macroscopic findings observed at the end of the recovery phase were limited to large draining lymph nodes (abnormal size, enlarged) in 1 male administered BNT162b2 (V9) and 1 female administered BNT162b3c and large inguinal lymph nodes (abnormal size, enlarged) in 1 female administered BNT162b3c, indicating a partial recovery of these findings. Pale/dark and/or firm injection sites and enlarged spleen were not observed at the end of recovery phase in BNT162b2 (V9) or BNT162b3c administered males and females, indicating a complete recovery of these findings.

Test article-related microscopic findings noted at the end of the dosing phase including edema at the injection site, hepatocellular vacuolation in the liver, 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, indicating a complete recovery of these findings. Inflammation at the injection site was characterized by mostly lymphocytes and plasma cells with few neutrophils (indicating partial recovery) and no edema (full recovery). However, increased cellularity of the germinal centers 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 plasmablasts 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.

7. INTEGRATED SUMMARY AND DISCUSSION OF RESULTS

Intramuscular administration of BNT162b2 (V9) and BNT162b3c at 30 µg RNA/dose day once weekly for a total of 3 doses to Wistar Han rats was tolerated during the dosing phase without evidence of systemic toxicity, generated a SARS-CoV-2 neutralizing antibody

response, and produced nonadverse changes consistent with inflammatory and immune responses to vaccine administration.

At the conclusion of the dosing phase, test article-related responses to both vaccines were evident as transient edema (very slight to moderate) and erythema (very slight) at the injection site after each dose of BNT162b2 (V9) and BNT162b3c. Test article-related erythema and edema fully resolved prior to subsequent dose administration on Days 8 and 15 with findings generally resolved by 72 hours after the final dose administration (Recovery Phase Day 1). Transiently higher body temperature differences compared with concurrent controls were noted on Days 1 (up to +0.71°C), 8 (up to +1.26°C) and 15 (up to +1.09°C) post administration of BNT162b3c and on Days 1 (up to +0.54°C), 8 (up to +0.98°C), and 15 (up to +1.03°C) after administration of BNT162b2 (V9). Additional body temperature evaluations were not needed at 48 and 72 hours postdose as individual animal body temperatures were ≤40°C at 24 hours postdose.

Changes secondary to inflammation included lower mean body weight (0.93x-0.94x control on Days 11 and 15) in male animals administered BNT162b3c and lower mean food consumption (0.83x-0.87x control on Days 4 and 11) for animals administered BNT162b2 (V9) and BNT162b3c (0.76x-0.92x control on Days 4 and 11) during the dosing phase. These changes fully resolved in the recovery phase as higher mean body weight (1.05-1.06x control) was noted in males only administered BNT162b2 (V9). Additionally, higher mean food consumption (1.08x-1.35x control) was noted throughout the recovery phase for male animals administered BNT162b2 (V9) and BNT162b3c (1.08x-1.30x control).

At the conclusion of the dosing phase, all clinical pathology findings (type and magnitude) 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 phase proteins (alpha-1 acid glycoprotein; 7.0x-42x controls], alpha-2-macroglobulin (3.3x-128x] and fibrinogen [2.4x-2.6x]) and white blood cell count (1.28x-2.95x; primarily involving neutrophils, monocytes and large unstained cells, which typically represent large mononuclear cells) and lower albumin:globulin (0.90x-0.82x). Hypersegmented neutrophils present on peripheral blood smears were considered 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). Collectively, these findings were consistent with immune responses to vaccines. Microscopic correlates included 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 centers of lymphoid organs. In addition, there were transiently lower reticulocyte counts on Day 4 (0.44x-0.27x), and higher reticulocytes on Day 17 (1.20x-1.31x; females only), with minor lower red cell mass on Days 4 and 17 (HCT; 0.93x-0.89x). Lower reticulocytes were interpreted to be a transient effect of innate immune responses (Abreu et al, 2018; Brooks et al, 2017; Kim et al, 2014; Wrighting & Andrews, 2006).

All test article-related clinical pathology parameter changes were fully reversed after a 3-week recovery phase, with the exception of higher red cell distribution width in males and

females administered BNT162b2(V9) (1.13x and 1.21x, respectively) and BNT162b3c (1.12x and 1.23x, respectively), higher globulins in males administered BNT162b2(V9) (1.08x) and females administered BNT162b2(V9) (1.06x) and BNT162b3c (1.07x) and lower AG ratio in females administered BNT162b2(V9) (0.91x).

Test article-related microscopic pathology findings were observed at the injection site and in the lymph nodes, spleen, bone marrow, and liver for both vaccine candidates. All microscopic findings were nonadverse, as there was no evidence of systemic toxicity or clinical signs of illness or lameness.

At the end of the dosing phase, test article-related mixed cell inflammation (mild to moderate) and edema (mild to moderate) at the injection site were consistent with findings typically associated with the IM administration of lipid nanoparticle (LNP)-encapsulated mRNA vaccines (Hassett et al, 2019). These findings correlated with macroscopic observations of abnormal color (dark/pale) and consistency (firm). At the end of the 3-week recovery phase, full recovery occurred for macroscopic findings of pale/dark and firm injection sites and the microscopic finding of edema, whereas partial recovery occurred for inflammation at the injection sites.

At the end of the dosing phase, test article-related findings in the lymph nodes (increased cellularity of plasma cells [minimal to moderate] and germinal centers [minimal to mild]), spleen (increased cellularity of hematopoietic cells [minimal] and germinal centers [minimal]), and the bone marrow (minimal increased cellularity of hematopoietic cells) were secondary to immune activation and/or inflammation at the injection site. The presence of plasma cells (interpreted as plasmablasts) in the draining and inguinal lymph nodes was interpreted to reflect a robust immunological response to the vaccines. These observations correlated with macroscopic observations of abnormal size (enlarged) in the lymph nodes and spleen and increased spleen weights. At the end of the 3-week recovery phase, full recovery occurred for higher spleen weights, macroscopic finding of enlarged spleen, and microscopic findings of increased cellularity of hematopoietic cells in the spleen and bone marrow, whereas partial recovery occurred for macroscopic findings of enlarged draining and inguinal lymph nodes, microscopic findings of increased cellularity of plasma cells and germinal centers in the draining and inguinal lymph nodes, and increased cellularity of the germinal centers in the spleen.

At the end of the dosing phase, test article-related microscopic finding of minimal portal hepatocyte vacuolation was not associated with hepatic tissue damage or liver enzyme alterations. This change may be related to hepatic clearance of the pegylated lipid in the LNP (Ivens et al, 2015). At the end of 3-week recovery phase, this finding was completely recovered.

Administration of 3 once weekly doses of BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses in both males and females at the end of the dosing (Day 17) and recovery phases (Day 21) of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals.

There were no other test article-related effects in the study.

8. CONCLUSIONS

In conclusion, BNT162b2 (V9) and BNT162b3c administered via intramuscular injection once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats was tolerated without evidence of systemic toxicity, generated a SARS-CoV-2 neutralizing antibody response, and produced nonadverse changes consistent with an immune or inflammatory response at the conclusion of the dosing phase. At the end of the 3-week recovery phase, full or partial recovery of all findings was observed. Other nonadverse findings included vacuolation in the liver which may be related to hepatic clearance of PEGylated lipids and was noted at the conclusion of the dosing phase and completely recovered. The findings in this study are consistent with those typically associated with the intramuscular administration of LNP-encapsulated mRNA vaccines. Animals administered BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses at the end of the dosing and recovery phases of the study.

9. REFERENCES

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Table 1

Clinical Signs - Daily Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Animals were considered normal (data not displayed in table) unless indicated otherwise.

PID = Prior to Initiation of Dosing

- = Value not applicable.

Table 1

Clinical Signs - Daily Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Males (PID)

Group Number:	1		2		3			
Dose:	0 µg/day		30 µg/day		30 µg /day			
Number of animals:	15		15		15			
Number Examined:	15		15		15			
Number Normal:	15		14		15			
Observations	a	b	a	b	a	b		
Tail Crooked	0	0	1	12	0	0		

Note: a = Number of animals with Observation
b = Number of days Observation seen

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Table 1

Clinical Signs - Daily Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Males (Dosing)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	14		13		15	
Observations	a	b	a	b	a	b
Tail Crooked	0	0	1	17	0	0
Thin Appearance	1	1	0	0	0	0
Hair Loss	0	0	1	2	0	0

Note: a = Number of animals with Observation
b = Number of days Observation seen

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Table 1

Clinical Signs - Daily Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Males (Recovery)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	5		5		5	
Number Normal:	5		4		5	
Observations	a	b	a	b	a	b
Tail Crooked	0	0	1	22	0	0

Note: a = Number of animals with Observation
b = Number of days Observation seen

Table 1

Clinical Signs - Daily Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Females (Dosing)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	15		14		14	
Observations	a	b	a	b	a	b
Lesion	0	0	0	0	1	1
Hair Loss	0	0	1	1	0	0

Note: a = Number of animals with Observation
b = Number of days Observation seen

Table 2

Ocular Exam Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Animals were considered normal (data not displayed in table) unless indicated otherwise.

PID = Prior to Initiation of Dosing

- = Value not applicable.

Table 2

Ocular Exam Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Males (PID)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	0		0		0	
Observations	a	b	a	b	a	b
Keratic Precipitates	1	1	0	0	1	1
No Ocular Abnormality	11	1	15	1	14	1
Retina, Tortuous Vessels	1	1	0	0	0	0
Vitreous, Hemorrhage	1	1	0	0	0	0
Vitreous, Hyaloid Remnant	1	1	0	0	0	0

Note: a = Number of animals with Observation
b = Number of days Observation seen

Table 2

Ocular Exam Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Males (Dosing)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	0		0		0	
Observations	a	b	a	b	a	b
Keratic Precipitates	1	1	0	0	1	1
No Ocular Abnormality	11	1	15	1	14	1
Retina, Tortuous Vessels	1	1	0	0	0	0
Vitreous, Hemorrhage	1	1	0	0	0	0
Vitreous, Hyaloid Remnant	1	1	0	0	0	0

Note: a = Number of animals with Observation
b = Number of days Observation seen

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Table 2

Ocular Exam Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Females (PID)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	0		0		0	
Observations	a	b	a	b	a	b
No Ocular Abnormality	15	1	15	1	15	2

Note: a = Number of animals with Observation
b = Number of days Observation seen

Table 2

Ocular Exam Summary Report by Interval

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Females (Dosing)

Group Number:	1		2		3	
Dose:	0 µg/day		30 µg/day		30 µg /day	
Number of animals:	15		15		15	
Number Examined:	15		15		15	
Number Normal:	0		0		0	
Observations	a	b	a	b	a	b
Keratic Precipitates	1	1	0	0	0	0
No Ocular Abnormality	14	1	15	1	15	1

Note: a = Number of animals with Observation
b = Number of days Observation seen

Table 3

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

N = Sample Size; SD = Standard Deviation; - = Value not applicable;
@ = Number examined reduced due to excluded data; e = Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical tests;
* = Statistically significant pairwise comparison at 0.05 level;
† = Statistically significant pairwise comparison at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level.
+ = Ascending trend sign;
- = Descending trend sign;

Table 3

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

	Group Number:		REF		2			3			
	Dose:		0 µg/day		30 µg/day			30 µg /day			
Phase	**Day**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	
PID	1	15	187.71	8.56	15	188.41	7.75	15	188.03	6.62	
	6	15	225.28	9.66	15	226.59	8.47	15	225.85	8.67	
Dosing	1	15	264.80	11.89	15	267.18	8.15	15	263.46	12.10	
	4	15	252.16	10.99	15	247.61	10.02	15	242.54	13.20	
	8	15	280.60	25.91	15	283.61	12.16	15	276.29	15.86	
	11	15	295.83	17.57	15	283.71	13.88	15	274.58	18.39	†
	15	15	311.47	17.82	15	302.53	15.32	15	293.29	17.38	*
Recovery	1	5	307.70	21.74	5	308.50	12.01	5	295.92	9.49	
	4	5	316.08	25.11	5	320.72	13.14	5	306.16	9.09	
	8	5	326.54	29.34	5	332.88	15.20	5	320.72	10.07	
	11	5	330.74	30.51	5	346.54	14.64	5	327.60	8.95	
	15	5	333.60	32.63	5	354.64	18.28	5	334.80	12.51	
	18	5	341.42	35.91	5	359.48	16.87	5	344.14	12.32	
	21	5	347.88	39.32	5	369.60	21.74	5	354.24	11.39	

Table 3

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

	Group Number:		**REF**			**2**			**3**	
	Dose:		**0 µg/day**			**30 µg/day**			**30 µg /day**	
Phase	**Day**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
PID	1	15	158.37	7.52	15	159.83	7.42	15	159.57	6.72
	6	15	176.36	7.52	15	176.31	7.51	15	175.61	9.68
Dosing	1	15	194.79	8.63	15	191.53	8.38	15	192.68	9.71
	4	15	183.19	8.90	15	177.31	6.25	15	176.93	7.46
	8	15	206.53	11.91	15	202.51	7.98	15	198.91	12.14
	11	15	210.23	12.88	15	203.88	8.25	15	202.83	11.29
	15	15	214.29	11.95	15	214.02	11.69	15	213.93	14.12
Recovery	1	5	215.08	14.40	5	207.22	4.75	5	211.92	22.04
	4	5	217.14	16.97	5	213.00	7.23	5	214.38	17.62
	8	5	224.02	20.44	5	220.14	7.28	5	219.88	17.62
	11	5	224.02	17.73	5	221.50	7.28	5	218.22	15.76
	15	5	224.24	13.98	5	220.58	5.81	5	217.30	19.01
	18	5	225.54	15.89	5	224.56	7.07	5	225.18	20.90
	21	5	228.86	14.34	5	231.32	10.43	5	224.46	18.18

Table 4

Body Weight Change During Interval (g)
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

N = Sample Size; SD = Standard Deviation; - = Value not applicable;
@= Number examined reduced due to excluded data; e= Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical tests;
* = Statistically significant pairwise comparison at 0.05 level;
† = Statistically significant pairwise comparison at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level;
+ = Ascending trend sign;
- = Descending trend sign;

Table 4

Body Weight Change During Interval (g)
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

	Group Number:		REF			2			3		
	Dose:		0 µg/day			30 µg/day			30 µg /day		
Phase	**Days**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	
PID	1-6	15	37.57	5.03	15	38.17	3.68	15	37.81	5.25	
Dosing	1-4	15	-12.64	6.48	15	-19.57	4.15 †	15	-20.92	5.13	†
	4-8	15	28.44	20.74	15	36.01	5.43	15	33.75	6.25	
	8-11	15	15.23	13.92	15	0.10	4.24 †	15	-1.71	4.92	†
	11-15	15	15.64	6.06	15	18.82	3.78 *	15	18.71	3.81	
	1-15	15	46.67	11.76	15	35.35	9.13 †	15	29.83	7.68	†
Recovery	1-4	5	8.38	6.59	5	12.22	3.59	5	10.24	1.50	
	4-8	5	10.46	5.99	5	12.16	3.36	5	14.56	2.43	
	8-11	5	4.20	2.25	5	13.66	5.19 †	5	6.88	2.09	
	11-15	5	2.86	5.01	5	8.10	4.39	5	7.20	4.36	
	15-18	5	7.82	4.23	5	4.84	2.74	5	9.34	1.78	
	18-21	5	6.46	3.71	5	10.12	5.61	5	10.10	4.17	
	1-21	5	40.18	23.53	5	61.10	11.09	5	58.32	2.92	

Table 4

Body Weight Change During Interval (g)
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number:		REF			2			3		
Dose:		0 µg/day			30 µg/day			30 µg /day		
Phase	**Days**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
PID	1-6	15	17.99	2.73	15	16.48	4.42	15	16.03	5.51
Dosing	1-4	15	-11.61	4.28	15	-14.21	4.68	15	-15.75	4.41
	4-8	15	23.34	6.05	15	25.19	3.75	15	21.98	6.34
	8-11	15	3.71	6.72	15	1.37	5.88	15	3.92	6.86
	11-15	15	4.06	2.94	15	10.14	5.89 †	15	11.09	7.60 †
	1-15	15	19.50	10.28	15	22.49	7.98	15	21.25	9.62
Recovery	1-4	5	2.06	4.97	5	5.78	7.47	5	2.46	8.25
	4-8	5	6.88	5.44	5	7.14	3.16	5	5.50	2.38
	8-11	5	0.00	6.15	5	1.36	4.33	5	-1.66	4.65
	11-15	5	0.22	5.29	5	-0.92	3.88	5	-0.92	7.60
	15-18	5	1.30	3.45	5	3.98	3.54	5	7.88	2.12 †
	18-21	5	3.32	6.18	5	6.76	7.21	5	-0.72	6.12
	1-21	5	13.78	7.24	5	24.10	9.09	5	12.54	9.04

Table 5

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

N = Sample Size; SD = Standard Deviation; -= Value not applicable;
@= Number examined reduced due to excluded data; e= Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical tree;
* = Statistically significant pairwise comparison at 0.05 level;
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‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level;
+ = Ascending trend sign;
- = Descending trend sign;

Table 5

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

	Group Number:		REF			2				3			
	Dose:		0 µg/day			30 µg/day				30 µg /day			
Phase	**Days**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**		**N**	**Mean**	**SD**		
Dosing	1-4	15	50.88	4.05	15	42.59	4.28	†	15	38.69	5.13	†
	4-8	15	90.87	21.27	15	96.75	8.55		15	91.37	11.71	
	8-11	15	64.77	5.09	15	54.02	6.13	†	15	50.45	7.45	†
	11-15	15	89.35	5.58	15	92.22	8.57		15	88.80	8.32	
	1-15	15	295.87	26.49	15	285.59	25.00		15	269.31	27.34	*
Recovery	1-4	5	48.02	6.41	5	64.74	3.38	†	5	62.26	4.67	†
	4-8	5	82.12	11.18	5	92.92	7.90		5	86.64	6.45	
	8-11	5	58.12	6.67	5	68.00	5.18	*	5	62.70	4.15	
	11-15	5	76.42	9.49	5	84.72	7.87		5	79.20	6.20	
	15-18	5	59.70	8.18	5	64.46	3.81		5	62.60	5.08	
	18-21	5	59.28	9.02	5	66.44	4.09		5	62.14	7.53	
	1-21	5	383.66	49.21	5	441.28	29.93		5	415.54	31.74	

Table 5

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

	Group Number:		REF			2			3		
	Dose:		0 µg/day			30 µg/day			30 µg /day		
Phase	**Days**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**		**N**	**Mean**	**SD**	
Dosing	1-4	15	37.79	4.28	15	33.02	3.62	†	15	34.63	12.73	†
	4-8	15	74.46	8.29	15	70.83	4.58		15	71.73	6.76	
	8-11	15	48.27	6.64	15	41.85	2.97	†	15	40.42	6.17	†
	11-15	15	65.27	7.36	15	66.59	6.62		15	68.50	8.71	
	1-15	15	225.80	24.33	15	212.29	13.55		15	215.28	24.59	
Recovery	1-4	5	47.60	5.58	5	49.72	4.93		5	49.02	5.33	
	4-8	5	63.32	7.66	5	66.68	2.93		5	66.88	9.55	
	8-11	5	46.32	4.38	5	46.70	3.76		5	42.72	6.58	
	11-15	5	59.08	7.68	5	60.98	4.18		5	57.88	11.68	
	15-18	5	42.44	5.72	5	43.64	5.88		5	44.76	7.31	
	18-21	5	45.00	4.09	5	46.80	4.13		5	44.76	6.43	
	1-21	5	303.76	32.65	5	314.52	18.16		5	306.02	44.46	

Table 6

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
RBC	Red Blood Cells
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Conc
RDW	Red Cell Distribution Width
RETIC	Reticulocyte, Absolute
PLT	Platelets
MPV	Mean Platelet Volume
WBC	White Blood Cells
NEUT	Neutrophil, Absolute
LYM	Lymphocyte, Absolute
MONO	Monocyte, Absolute
EO	Eosinophil, Absolute
BASO	Basophil, Absolute
LUC	Large Unstained Cells, Absolute
PT_Rat	Prothrombin Time, Rat
APTT	Activated Partial Thromboplastin Time
FIB	Fibrinogen

Table 6

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Sample size is displayed in ( ) before the mean value.
SD = Standard Deviation; - = Value not applicable;
Units are displayed in the ( ) under each parameter name;
HPD = Hours Post Dose; U = Unscheduled;
e = Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical test;
* = Statistically significant pairwise comparison at 0.05 level;
† = Statistically significant pairwise comparison at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level.
+ = Ascending trend sign;
- = Descending trend sign;
= Individual parameter values reported as less than or greater than limit of quantitation are set equal to the limit to calculate the average.

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
RBC (10^6/uL)	Dosing	4	--	**Mean**	(7) 8.117		(7) 7.774	*	(7) 7.596	†
				SD	0.265		0.292		0.273	
		17	-	**Mean**	(9) 7.584		(10) 7.169		(10) 7.113	
				SD	0.512		0.292		0.326	
	Recovery	22	-	**Mean**	(5) 7.950		(5) 8.064		(5) 7.886	
				SD	0.480		0.261		0.427	
HGB (g/dL)	Dosing	4	--	**Mean**	(7) 15.01		(7) 14.16	*	(7) 14.01	†
				SD	0.57		0.62		0.38	
		17	-	**Mean**	(9) 13.82		(10) 12.53	†	(10) 12.81	†
				SD	0.72		0.63		0.49	
	Recovery	22	-	**Mean**	(5) 14.36		(5) 14.38		(5) 14.00	
				SD	1.02		0.41		0.45	
HCT (%)	Dosing	4	--	**Mean**	(7) 48.04		(7) 43.37	†	(7) 43.79	†
				SD	1.33		1.69		1.16	
		17	-	**Mean**	(9) 42.61		(10) 38.40	†	(10) 39.29	*
				SD	2.44		1.64		1.49	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
HCT (%)	Recovery	22	-	Mean	(5) 42.78		(5) 43.72		(5) 42.98	
				SD	3.12		1.44		2.00	
MCV (fL)	Dosing	4	--	Mean	(7) 59.19		(7) 55.81	†	(7) 57.69	
				SD	1.21		1.28		1.52	
		17	-	Mean	(9) 56.24		(10) 53.58	†	(9) 54.99	
				SD	1.37		1.36		1.35	
	Recovery	22	-	Mean	(4) 53.80		(4) 54.00		(4) 54.30	
				SD	1.15		1.03		1.25	
MCH (pg)	Dosing	4	--	Mean	(7) 18.51		(7) 18.20		(7) 18.50	
				SD	0.48		0.49		0.47	
		17	-	Mean	(9) 18.27		(10) 17.48	†	(10) 18.01	
				SD	0.42		0.51		0.60	
	Recovery	22	-	Mean	(5) 18.06		(5) 17.84		(5) 17.80	
				SD	0.43		0.64		0.66	
MCHC (g/dL)	Dosing	4	--	Mean	(7) 31.24		(7) 32.64	†	(7) 32.04	†
				SD	0.57		0.40		0.26	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
MCHC (g/dL)	Dosing	17	-	Mean	(9) 32.46		(10) 32.65		(10) 32.61	
				SD	0.36		0.53		0.64	
	Recovery	22	-	Mean	(5) 33.60		(5) 32.90		(5) 32.60	*
				SD	0.71		0.74		0.63	
RDW (%)	Dosing	4	--	Mean	(7) 12.27		(7) 12.83		(7) 12.44	
				SD	0.47		0.70		0.49	
		17	-	Mean	(9) 11.63		(10) 14.12	†	(9) 13.73	†
				SD	0.39		0.73		0.46	
	Recovery	22	-	Mean	(4) 11.93		(4) 13.48	†	(4) 13.33	*
				SD	0.42		0.29		0.46	
RETIC (10^3/uL)	Dosing	4	--	Mean	(7) 392.1		(7) 107.4	†	(7) 104.6	†
				SD	51.5		46.9		27.3	
		17	-	Mean	(9) 178.8		(10) 185.4		(10) 194.0	
				SD	24.1		25.9		12.4	
	Recovery	22	-	Mean	(5) 180.8		(5) 190.8		(5) 186.6	
				SD	28.9		30.4		25.4	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
PLT (10^3/uL)	Dosing	4	--	Mean	(7)	1012.7	(7)	1039.7		(7)	945.1	
				SD		169.9		94.7			132.1	
		17	-	Mean	(9)	881.3	(10)	801.3		(10)	739.0	*
				SD		69.0		119.9			133.1	
	Recovery	22	-	Mean	(5)	847.8	(5)	904.4		(5)	837.6	
				SD		40.0		115.4			115.3	
MPV (fL)	Dosing	4	--	Mean	(7)	8.87	(7)	9.14		(7)	9.70	†
				SD		0.35		0.71			0.37	
		17	-	Mean	(9)	9.12	(10)	9.55		(10)	9.93	†
				SD		0.36		0.47			0.51	
	Recovery	22	-	Mean	(5)	9.00	(5)	8.84		(5)	8.88	
				SD		0.23		0.24			0.26	
WBC (10e3/uL)	Dosing	4	--	Mean	(7)	7.60	(7)	10.70	*	(7)	9.70	
				SD		1.08		3.01			1.64	
		17	-	Mean	(9)	3.84	(10)	8.83	†	(10)	8.60	†
				SD		1.67		3.62			1.15	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	**REF**			**2**			**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**			**30 µg/day**			**30 µg /day**	
WBC (10e3/uL)	Recovery	22	-	**Mean**	(5)	5.26	(5)	5.98		(5)	4.90	
				SD		2.64		1.16			1.18	
NEUT (10^3/uL)	Dosing	4	--	**Mean**	(7)	1.083	(7)	2.470	†	(7)	2.161	*
				SD		0.420		0.834			0.521	
		17	-	**Mean**	(9)	0.674	(10)	4.449	†	(10)	4.351	†
				SD		0.387		1.890			0.696	
	Recovery	22	-	**Mean**	(5)	0.898	(5)	1.070		(5)	1.276	
				SD		0.372		0.215			0.329	
LYM (10^3/uL)	Dosing	4	--	**Mean**	(7)	6.284	(7)	7.727		(7)	7.030	
				SD		1.048		2.157			1.150	
		17	-	**Mean**	(9)	3.009	(10)	3.792		(10)	3.547	
				SD		1.282		1.624			0.574	
	Recovery	22	-	**Mean**	(5)	4.158	(5)	4.672		(5)	3.408	
				SD		2.205		1.107			0.839	
MONO (10^3/uL)	Dosing	4	--	**Mean**	(7)	0.109	(7)	0.199	*	(7)	0.214	†
				SD		0.021		0.079			0.022	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
MONO (10^3/uL)	Dosing	17	-	Mean	(9) 0.071		(10) 0.234	†	(10) 0.254	†
				SD	0.042		0.121		0.077	
	Recovery	22	-	Mean	(5) 0.074		(5) 0.106		(5) 0.104	
				SD	0.031		0.021		0.021	
EO (10^3/uL)	Dosing	4	--	Mean	(7) 0.081		(7) 0.086		(7) 0.091	
				SD	0.059		0.054		0.034	
		17	-	Mean	(9) 0.056		(10) 0.141	†	(10) 0.122	†
				SD	0.024		0.053		0.061	
	Recovery	22	-	Mean	(5) 0.068		(5) 0.074		(5) 0.074	
				SD	0.042		0.024		0.038	
BASO (10^3/uL)	Dosing	4	--	Mean	(7) 0.016		(7) 0.030	*	(7) 0.037	†
				SD	0.005		0.014		0.014	
		17	-	Mean	(9) 0.003		(10) 0.017	†	(10) 0.019	†
				SD	0.005		0.013		0.007	
	Recovery	22	-	Mean	(5) 0.008		(5) 0.008		(5) 0.008	
				SD	0.013		0.004		0.004	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
LUC (10^3/uL)	Dosing	4	--	Mean	(7) 0.046		(7) 0.187	†		(7) 0.183	†	
				SD	0.011		0.139			0.104		
		17	-	Mean	(9) 0.026		(10) 0.209	†		(10) 0.323	†	
				SD	0.013		0.145			0.118		
	Recovery	22	-	Mean	(5) 0.034		(5) 0.048			(5) 0.026		
				SD	0.027		0.011			0.009		
PT_Rat (sec)	Dosing	17	-	Mean	(8) 14.64		(9) 15.63	*		(10) 16.35	†	
				SD	0.76		1.20			0.71		
	Recovery	22	-	Mean	(5) 15.34		(5) 16.64			(5) 18.68	*	
				SD	1.30		1.51			1.78		
APTT (sec)	Dosing	17	-	Mean	(8) 14.41		(9) 16.50	*		(10) 16.78	*	
				SD	1.81		2.65			1.78		
	Recovery	22	-	Mean	(5) 16.44		(5) 17.76	*		(5) 18.12	†	
				SD	0.50		0.79			0.67		
FIB (mg/dL)	Dosing	17	-	Mean	(8) 253.1		(9) 596.7	†		(10) 606.1	†	
				SD	14.3		39.6			53.9		

Table 6

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
FIB (mg/dL)	Recovery	22	-	**Mean**	(5)	264.8	(5)	266.6	(5)	264.0
				SD		30.7		21.9		10.8

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**			**3**		
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**			**30 µg /day**		
RBC (10^6/uL)	Dosing	4	--	**Mean**	(7)	7.903	(7)	7.381	*	(7)	7.470	*
				SD		0.370		0.190			0.206	
		17	-	**Mean**	(10)	7.423	(9)	6.872	†	(7)	6.836	†
				SD		0.183		0.195			0.343	
	Recovery	22	-	**Mean**	(5)	7.262	(5)	7.838	†	(5)	7.704	*
				SD		0.267		0.256			0.208	
HGB (g/dL)	Dosing	4	--	**Mean**	(7)	14.53	(7)	13.56	*	(7)	13.56	*
				SD		0.59		0.62			0.58	
		17	-	**Mean**	(10)	13.83	(9)	12.38	†	(7)	12.24	†
				SD		0.31		0.34			0.68	
	Recovery	22	-	**Mean**	(5)	13.64	(5)	13.92		(5)	14.14	
				SD		0.67		0.37			0.57	
HCT (%)	Dosing	4	--	**Mean**	(7)	44.91	(7)	41.79	*	(7)	41.81	*
				SD		1.91		1.79			1.29	
		17	-	**Mean**	(10)	41.67	(9)	38.09	†	(7)	37.21	†
				SD		0.70		0.98			1.75	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
HCT (%)	Recovery	22	-	**Mean**	(5) 40.78		(5) 42.46		(5) 42.98	
				SD	1.82		0.93		1.99	
MCV (fL)	Dosing	4	--	**Mean**	(7) 56.84		(7) 56.59		(7) 56.03	
				SD	0.87		1.75		1.91	
		17	-	**Mean**	(10) 56.16		(9) 55.43		(6) 54.40	
				SD	1.19		1.71		1.97	
	Recovery	22	-	**Mean**	(4) 55.80		(5) 54.22		(5) 55.78	
				SD	2.62		1.55		2.21	
MCH (pg)	Dosing	4	--	**Mean**	(7) 18.37		(7) 18.39		(7) 18.16	
				SD	0.22		0.67		0.75	
		17	-	**Mean**	(10) 18.62		(9) 17.99	†	(7) 17.89	†
				SD	0.35		0.49		0.60	
	Recovery	22	-	**Mean**	(5) 18.78		(5) 17.76		(5) 18.38	
				SD	0.97		0.38		0.82	
MCHC (g/dL)	Dosing	4	--	**Mean**	(7) 32.34		(7) 32.49		(7) 32.41	
				SD	0.30		0.78		0.63	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

	Phase	Day	HPD	Group Number: / Dose :	REF 0 µg/day		2 30 µg/day		3 30 µg /day	
MCHC (g/dL)	Dosing	17	-	Mean	(10) 33.18		(9) 32.50	†	(7) 32.84	
				SD	0.32		0.41		0.59	
	Recovery	22	-	Mean	(5) 33.46		(5) 32.78		(5) 32.96	
				SD	0.56		0.33		0.75	
RDW (%)	Dosing	4	--	Mean	(7) 11.11		(7) 11.39		(7) 11.97	†
				SD	0.29		0.40		0.68	
		17	-	Mean	(10) 11.33		(9) 13.34	†	(6) 13.38	†
				SD	0.43		1.04		0.64	
	Recovery	22	-	Mean	(4) 10.80		(5) 13.04	†	(5) 13.32	†
				SD	0.33		0.23		0.50	
RETIC (10^3/uL)	Dosing	4	--	Mean	(7) 301.7		(7) 129.7	†	(7) 133.6	†
				SD	39.4		35.7		39.1	
		17	-	Mean	(10) 168.9		(9) 222.1	*	(7) 203.3	
				SD	34.7		54.7		45.8	
	Recovery	22	-	Mean	(5) 153.2		(5) 155.0		(5) 136.2	
				SD	36.2		16.0		49.9	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
PLT (10^3/uL)	Dosing	4	--	Mean	(7)	927.1	(7)	1003.9		(7)	973.6	
				SD		116.6		70.9			168.1	
		17	-	Mean	(10)	906.9	(9)	778.0	*	(7)	757.6	
				SD		124.5		88.3			151.1	
	Recovery	22	-	Mean	(5)	787.6	(5)	838.2		(5)	782.0	
				SD		77.7		88.0			56.6	
MPV (fL)	Dosing	4	--	Mean	(7)	8.67	(7)	8.91		(7)	8.99	
				SD		0.91		0.25			0.81	
		17	-	Mean	(10)	9.50	(9)	9.40		(7)	9.73	
				SD		0.49		0.21			0.72	
	Recovery	22	-	Mean	(5)	9.20	(5)	9.02		(5)	9.18	
				SD		0.42		0.34			0.33	
WBC (10e3/uL)	Dosing	4	--	Mean	(7)	6.01	(7)	7.84		(7)	8.57	*
				SD		2.38		1.98			0.92	
		17	-	Mean	(10)	2.16	(9)	5.70	†	(7)	6.37	†
				SD		0.45		1.33			2.46	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF			2			3		
	Phase	Day	HPD	Dose :	0 µg/day			30 µg/day			30 µg /day		
WBC (10e3/uL)	Recovery	22	-	Mean	(5)	2.34		(5)	2.62		(5)	2.72	
				SD		0.87			0.69			1.16	
NEUT (10^3/uL)	Dosing	4	--	Mean	(7)	0.920		(7)	2.306		(7)	2.879	†
				SD		1.220			0.683			0.478	
		17	-	Mean	(10)	0.409		(9)	2.469	†	(7)	2.879	†
				SD		0.198			0.711			1.238	
	Recovery	22	-	Mean	(5)	0.252		(5)	0.482	*	(5)	0.278	
				SD		0.051			0.279			0.051	
LYM (10^3/uL)	Dosing	4	--	Mean	(7)	4.911		(7)	5.136		(7)	5.169	
				SD		1.263			1.368			0.932	
		17	-	Mean	(10)	1.651		(9)	2.833	†	(7)	3.030	†
				SD		0.289			0.872			1.209	
	Recovery	22	-	Mean	(5)	2.016		(5)	2.050		(5)	2.316	
				SD		0.899			0.554			1.068	
MONO (10^3/uL)	Dosing	4	--	Mean	(7)	0.093		(7)	0.176		(7)	0.234	†
				SD		0.092			0.054			0.062	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
MONO (10^3/uL)	Dosing	17	-	**Mean**	(10) 0.056		(9) 0.154	†	(7) 0.176	†
				SD	0.025		0.033		0.068	
	Recovery	22	-	**Mean**	(5) 0.028		(5) 0.048		(5) 0.060	*
				SD	0.013		0.011		0.025	
EO (10^3/uL)	Dosing	4	--	**Mean**	(7) 0.057		(7) 0.087	*	(7) 0.123	†
				SD	0.011		0.023		0.039	
		17	-	**Mean**	(10) 0.029		(9) 0.092	†	(7) 0.097	†
				SD	0.013		0.043		0.042	
	Recovery	22	-	**Mean**	(5) 0.032		(5) 0.028		(5) 0.036	
				SD	0.011		0.011		0.021	
BASO (10^3/uL)	Dosing	4	--	**Mean**	(7) 0.009		(7) 0.017		(7) 0.024	†
				SD	0.007		0.010		0.005	
		17	-	**Mean**	(10) 0.001		(9) 0.008	†	(7) 0.010	†
				SD	0.003		0.004		0.006	
	Recovery	22	-	**Mean**	(5) 0.000		(5) 0.000		(5) 0.002	
				SD	0.000		0.000		0.004	

Table 6

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**			**3**		
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**			**30 µg /day**		
LUC (10^3/uL)	Dosing	4	--	**Mean**	(7)	0.030	(7)	0.126	†	(7)	0.133	†
				SD		0.028		0.093			0.060	
		17	-	**Mean**	(10)	0.010	(9)	0.132	†	(7)	0.190	†
				SD		0.005		0.101			0.096	
	Recovery	22	-	**Mean**	(5)	0.014	(5)	0.012		(5)	0.022	
				SD		0.005		0.008			0.016	
PT_Rat (sec)	Dosing	17	-	**Mean**	(10)	14.12	(9)	14.89		(9)	15.38	*
				SD		0.84		1.02			0.93	
	Recovery	22	-	**Mean**	(5)	13.10	(5)	13.66		(5)	13.58	
				SD		0.83		0.83			0.62	
APTT (sec)	Dosing	17	-	**Mean**	(10)	15.45	(9)	15.56		(9)	14.78	
				SD		0.80		1.39			3.08	
	Recovery	22	-	**Mean**	(5)	16.82	(5)	17.26		(5)	16.96	
				SD		0.85		0.90			0.72	
FIB (mg/dL)	Dosing	17	-	**Mean**	(10)	217.2	(9)	541.9	†	(9)	563.1	†
				SD		25.0		63.4			56.7	

Table 6

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
FIB (mg/dL)	Recovery	22	-	**Mean**	(5)	186.4	(5)	196.6	(5)	185.0
				SD		17.2		18.4		16.6

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
GGT	Gamma Glutamyl Transferase
TBIL	Bilirubin, Total
CHOL	Cholesterol
TRIG	Triglycerides
GLUC	Glucose
TP	Protein, Total
ALB	Albumin
GLOB	Globulin
AG	Albumin/Globulin Ratio
BUN	Blood Urea Nitrogen
CREA	Creatinine
PHOS	Phosphorus
CA	Calcium
NA	Sodium
K	Potassium
CL	Chloride
A2M	Alpha-2-Macroglobulin

Parameter	Description
A1AGP	Alpha-1 Acid Glycoprotein

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Sample size is displayed in ( ) before the mean value.
SD = Standard Deviation; - = Value not applicable;
Units are displayed in the ( ) under each parameter name;
HPD = Hours Post Dose; U = Unscheduled;
e = Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical test;
* = Statistically significant pairwise comparison at 0.05 level;
† = Statistically significant pairwise comparison at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level.
+ = Ascending trend sign;
- = Descending trend sign;
= Individual parameter values reported as less than or greater than limit of quantitation are set equal to the limit to calculate the average.

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
ALT (U/L)	Dosing	4	---	Mean	(8)	29.1	(8)	33.3		(8)	28.8	
				SD		6.9		6.5			5.6	
		17	-	Mean	(10)	18.1	(10)	22.9	*	(10)	20.8	
				SD		2.4		4.7			3.0	
	Recovery	22	-	Mean	(5)	19.2	(5)	17.6		(5)	17.4	
				SD		3.3		2.5			3.0	
AST (U/L)	Dosing	4	---	Mean	(8)	94.5	(8)	103.1		(8)	97.8	
				SD		8.3		14.7			14.0	
		17	-	Mean	(10)	71.7	(10)	84.2	*	(10)	86.8	†
				SD		5.3		15.4			8.5	
	Recovery	22	-	Mean	(5)	91.8	(5)	94.0		(5)	97.0	
				SD		10.3		13.5			4.6	
ALP (U/L)	Dosing	4	---	Mean	(8)	166.6	(8)	195.4	*	(8)	188.3	
				SD		50.3		28.2			29.0	
		17	-	Mean	(10)	97.6	(10)	103.4		(10)	110.0	
				SD		25.9		18.9			22.8	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
ALP (U/L)	Recovery	22	-	**Mean**	(5)	84.4	(5)	79.6	(5)	83.4
				SD		17.0		9.3		18.6
GGT (U/L)	Dosing	4	---	**Mean**	(8)	3.0	(8)	3.0	(8)	3.0
				SD	#	0.0	#	0.0	#	0.0
		17	-	**Mean**	(10)	3.0	(10)	3.0	(10)	3.0
				SD	#	0.0	#	0.0	#	0.0
	Recovery	22	-	**Mean**	(5)	3.0	(5)	3.0	(5)	3.0
				SD	#	0.0	#	0.0	#	0.0
TBIL (mg/dL)	Dosing	4	---	**Mean**	(8)	0.10	(8)	0.10	(8)	0.10
				SD	#	0.00	#	0.00	#	0.00
		17	-	**Mean**	(10)	0.10	(10)	0.10	(10)	0.10
				SD	#	0.00	#	0.00	#	0.00
	Recovery	22	-	**Mean**	(5)	0.10	(5)	0.10	(5)	0.10
				SD	#	0.00	#	0.00	#	0.00
CHOL (mg/dL)	Dosing	4	---	**Mean**	(8)	63.0	(8)	52.5	(8)	51.8
				SD		9.3		7.2		15.3

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

	Phase	Day	HPD	Group Number: / Dose :	REF 0 µg/day		2 30 µg/day			3 30 µg /day		
CHOL (mg/dL)	Dosing	17	-	Mean	(10)	51.7	(10)	40.2	†	(10)	37.2	†
				SD		6.7		6.1			8.6	
	Recovery	22	-	Mean	(5)	52.8	(5)	61.0	*	(5)	56.2	
				SD		7.2		5.7			4.4	
TRIG (mg/dL)	Dosing	4	---	Mean	(8)	62.0	(8)	42.8		(8)	51.9	
				SD		25.8		10.2			19.6	
		17	-	Mean	(10)	58.8	(10)	33.6	†	(10)	35.9	†
				SD		16.6		7.2			10.3	
	Recovery	22	-	Mean	(5)	49.0	(5)	50.8		(5)	45.6	
				SD		18.4		15.1			16.0	
GLUC (mg/dL)	Dosing	4	---	Mean	(8)	111.3	(8)	98.1		(8)	100.0	
				SD		14.2		12.6			16.7	
		17	-	Mean	(10)	131.7	(10)	117.4		(10)	122.6	
				SD		17.0		17.0			23.9	
	Recovery	22	-	Mean	(5)	137.0	(5)	121.4		(5)	119.8	
				SD		30.1		23.7			16.1	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
TP (g/dL)	Dosing	4	---	Mean	(8)	6.10	(8)	5.90		(8)	5.85	
				SD		0.21		0.22			0.22	
		17	-	Mean	(10)	5.39	(10)	5.51		(10)	5.41	
				SD		0.30		0.36			0.34	
	Recovery	22	-	Mean	(5)	5.82	(5)	6.08	*	(5)	5.90	
				SD		0.16		0.11			0.14	
ALB (g/dL)	Dosing	4	---	Mean	(8)	3.98	(8)	3.71	†	(8)	3.68	†
				SD		0.14		0.15			0.14	
		17	-	Mean	(10)	3.50	(10)	3.43		(10)	3.38	
				SD		0.19		0.21			0.22	
	Recovery	22	-	Mean	(5)	3.72	(5)	3.82		(5)	3.72	
				SD		0.11		0.08			0.13	
GLOB (g/dL)	Dosing	4	---	Mean	(8)	2.13	(8)	2.19		(8)	2.18	
				SD		0.09		0.10			0.10	
		17	-	Mean	(10)	1.89	(10)	2.08	*	(10)	2.03	
				SD		0.12		0.18			0.13	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2			3		
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day			30 µg /day		
GLOB (g/dL)	Recovery	22	-	Mean	(5)	2.10	(5)	2.26	†	(5)	2.18	
				SD		0.07		0.05			0.04	
AG (None)	Dosing	4	---	Mean	(8)	1.88	(8)	1.70	†	(8)	1.69	†
				SD		0.07		0.08			0.06	
		17	-	Mean	(10)	1.85	(10)	1.65	†	(10)	1.65	†
				SD		0.05		0.08			0.05	
	Recovery	22	-	Mean	(5)	1.76	(5)	1.72		(5)	1.70	
				SD		0.05		0.04			0.07	
BUN (mg/dL)	Dosing	4	---	Mean	(8)	23.8	(8)	26.0		(8)	23.8	
				SD		5.0		4.0			2.7	
		17	-	Mean	(10)	18.8	(10)	18.6		(10)	19.9	
				SD		3.9		3.2			2.8	
	Recovery	22	-	Mean	(5)	17.0	(5)	17.2		(5)	16.4	
				SD		1.7		1.3			3.8	
CREA (mg/dL)	Dosing	4	---	Mean	(8)	0.31	(8)	0.29		(8)	0.26	*
				SD		0.04		0.04			0.05	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
CREA (mg/dL)	Dosing	17	-	Mean	(10)	0.25	(10)	0.25	(10)	0.27
				SD		0.05		0.07		0.05
	Recovery	22	-	Mean	(5)	0.28	(5)	0.28	(5)	0.28
				SD		0.04		0.04		0.04
PHOS (mg/dL)	Dosing	4	---	Mean	(8)	7.34	(8)	7.41	(8)	7.58
				SD		0.56		0.45		0.38
		17	-	Mean	(10)	8.72	(10)	8.11	(10)	8.01
				SD		0.75		0.58		0.92
	Recovery	22	-	Mean	(5)	6.56	(5)	6.86	(5)	6.82
				SD		1.15		0.46		0.72
CA (mg/dL)	Dosing	4	---	Mean	(8)	9.76	(8)	9.65	(8)	9.75
				SD		0.25		0.28		0.32
		17	-	Mean	(10)	9.86	(10)	9.82	(10)	9.59
				SD		0.34		0.35		0.30
	Recovery	22	-	Mean	(5)	9.44	(5)	9.44	(5)	9.48
				SD		0.11		0.29		0.27

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
NA (mmol/L)	Dosing	4	---	Mean	(8)	144.4	(8)	144.1	(8)	143.8
				SD		1.6		1.4		1.2
		17	-	Mean	(10)	144.0	(10)	142.3	(10)	142.7
				SD		1.2		1.9		1.9
	Recovery	22	-	Mean	(5)	142.4	(5)	142.6	(5)	143.4
				SD		0.5		0.9		0.9
K (mmol/L)	Dosing	4	---	Mean	(8)	4.45	(8)	4.55	(8)	4.66
				SD		0.31		0.18		0.29
		17	-	Mean	(10)	4.30	(10)	4.36	(10)	4.32
				SD		0.16		0.31		0.19
	Recovery	22	-	Mean	(5)	4.12	(5)	4.26	(5)	4.20
				SD		0.28		0.26		0.20
CL (mmol/L)	Dosing	4	---	Mean	(8)	102.4	(8)	102.0	(8)	101.3
				SD		2.8		0.9		1.3
		17	-	Mean	(10)	104.8	(10)	103.4	(10)	104.2
				SD		0.9		1.8		1.3

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
CL (mmol/L)	Recovery	22	-	Mean	(5) 105.6		(5) 106.0		(5) 106.4	
				SD	1.5		1.0		1.1	
A2M (ug/mL)	Dosing	4	---	Mean	(8) 113.4		(8) 2318.1	†	(8) 3911.6	†
				SD	228.9		922.4		2866.1	
		17	-	Mean	(10) 14.0		(10) 990.6	†	(10) 1794.2	†
				SD	3.3		730.0		1234.1	
	Recovery	22	-	Mean	(5) 8.0		(5) 19.4	*	(5) 16.2	†
				SD	1.9		14.3		2.3	
A1AGP (ug/mL)	Dosing	4	---	Mean	(8) 174.358		(8) 1642.265	†	(8) 2351.791	†
				SD	312.769		312.914		1053.465	
		17	-	Mean	(10) 47.672		(10) 1835.986	†	(10) 2021.083	†
				SD	12.664		372.467		673.967	
	Recovery	22	-	Mean	(5) 54.910		(5) 75.740		(5) 62.562	
				SD	20.556		26.083		16.549	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF		2		3	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
ALT (U/L)	Dosing	4	---	**Mean**	(8) 20.9		(8) 24.9		(8) 23.1	
				SD	4.3		4.3		5.2	
		17	-	**Mean**	(9) 11.3		(9) 13.9	†	(8) 16.5	†
				SD	1.3		2.1		3.3	
	Recovery	22	-	**Mean**	(5) 11.8		(5) 14.8		(5) 13.6	
				SD	2.2		2.2		1.8	
AST (U/L)	Dosing	4	---	**Mean**	(8) 81.8		(8) 96.1		(8) 91.3	
				SD	11.5		14.6		10.4	
		17	-	**Mean**	(9) 69.9		(9) 81.7		(8) 80.3	
				SD	18.3		15.9		18.0	
	Recovery	22	-	**Mean**	(5) 65.4		(5) 73.6		(5) 67.2	
				SD	8.2		10.2		4.4	
ALP (U/L)	Dosing	4	---	**Mean**	(8) 92.9		(8) 137.9	†	(8) 143.4	†
				SD	21.7		21.4		31.1	
		17	-	**Mean**	(9) 50.9		(9) 78.1	†	(8) 97.4	†
				SD	10.3		17.7		18.8	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF		2		3	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
ALP (U/L)	Recovery	22	-	**Mean**	(5)	34.0	(5)	37.0	(5)	29.0
				SD		6.2		6.4		7.6
GGT (U/L)	Dosing	4	---	**Mean**	(8)	3.0	(8)	3.0	(8)	3.0
				SD	#	0.0	#	0.0	#	0.0
		17	-	**Mean**	(9)	3.0	(9)	3.0	(8)	3.0
				SD	#	0.0	#	0.0	#	0.0
	Recovery	22	-	**Mean**	(5)	3.0	(5)	3.0	(5)	3.0
				SD	#	0.0	#	0.0	#	0.0
TBIL (mg/dL)	Dosing	4	---	**Mean**	(8)	0.10	(8)	0.10	(8)	0.10
				SD	#	0.00	#	0.00	#	0.00
		17	-	**Mean**	(9)	0.10	(9)	0.10	(8)	0.10
				SD	#	0.00	#	0.00	#	0.00
	Recovery	22	-	**Mean**	(5)	0.10	(5)	0.10	(5)	0.10
				SD	#	0.00	#	0.00	#	0.00
CHOL (mg/dL)	Dosing	4	---	**Mean**	(8)	45.6	(8)	47.3	(8)	56.6
				SD		13.4		12.3		12.4

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

	Phase	Day	HPD	Group Number: / Dose :	REF 0 µg/day		2 30 µg/day		3 30 µg /day	
CHOL (mg/dL)	Dosing	17	-	Mean	(9) 33.4		(9) 33.7		(8) 31.9	
				SD	11.5		6.3		4.2	
	Recovery	22	-	Mean	(5) 43.0		(5) 54.2		(5) 41.2	
				SD	13.0		18.9		8.6	
TRIG (mg/dL)	Dosing	4	---	Mean	(8) 36.8		(8) 29.4		(8) 34.5	
				SD	13.0		6.8		7.8	
		17	-	Mean	(9) 27.8		(9) 25.1		(8) 26.5	
				SD	8.4		5.1		5.1	
	Recovery	22	-	Mean	(5) 30.8		(5) 31.8		(5) 37.2	
				SD	8.7		3.7		7.9	
GLUC (mg/dL)	Dosing	4	---	Mean	(8) 102.5		(8) 89.1	†	(8) 87.1	†
				SD	8.4		8.2		5.9	
		17	-	Mean	(9) 111.4		(9) 99.7		(8) 99.5	
				SD	16.4		7.7		8.8	
	Recovery	22	-	Mean	(5) 119.4		(5) 107.6		(5) 118.0	
				SD	14.3		10.7		22.2	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
TP (g/dL)	Dosing	4	---	Mean	(8) 6.26		(8) 5.65	†	(8) 5.94	
				SD	0.35		0.17		0.23	
		17	-	Mean	(9) 5.44		(9) 4.98	†	(8) 4.96	†
				SD	0.32		0.21		0.29	
	Recovery	22	-	Mean	(5) 6.52		(5) 6.54		(5) 6.74	
				SD	0.37		0.21		0.30	
ALB (g/dL)	Dosing	4	---	Mean	(8) 4.16		(8) 3.56	†	(8) 3.73	†
				SD	0.23		0.09		0.14	
		17	-	Mean	(9) 3.60		(9) 3.07	†	(8) 3.09	†
				SD	0.19		0.11		0.14	
	Recovery	22	-	Mean	(5) 4.26		(5) 4.14		(5) 4.32	
				SD	0.32		0.11		0.19	
GLOB (g/dL)	Dosing	4	---	Mean	(8) 2.10		(8) 2.09		(8) 2.21	
				SD	0.14		0.08		0.10	
		17	-	Mean	(9) 1.84		(9) 1.91		(8) 1.88	
				SD	0.15		0.12		0.18	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**			**2**			**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**			**30 µg/day**			**30 µg /day**	
GLOB (g/dL)	Recovery	22	-	**Mean**	(5)	2.26	(5)	2.40		(5)	2.42	
				SD		0.11		0.10			0.13	
AG (None)	Dosing	4	---	**Mean**	(8)	1.98	(8)	1.71	†	(8)	1.69	†
				SD		0.07		0.04			0.04	
		17	-	**Mean**	(9)	1.96	(9)	1.61	†	(8)	1.66	†
				SD		0.12		0.06			0.12	
	Recovery	22	-	**Mean**	(5)	1.90	(5)	1.72	*	(5)	1.80	
				SD		0.16		0.04			0.07	
BUN (mg/dL)	Dosing	4	---	**Mean**	(8)	16.8	(8)	18.8		(8)	18.3	
				SD		1.9		4.2			2.5	
		17	-	**Mean**	(9)	17.0	(9)	18.9		(8)	20.0	
				SD		3.0		3.3			1.3	
	Recovery	22	-	**Mean**	(5)	16.6	(5)	18.4		(5)	18.2	
				SD		3.0		2.7			1.8	
CREA (mg/dL)	Dosing	4	---	**Mean**	(8)	0.31	(8)	0.23	†	(8)	0.25	*
				SD		0.04		0.05			0.05	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
CREA (mg/dL)	Dosing	17	-	**Mean**	(9)	0.27	(9)	0.22	(8)	0.21
				SD		0.07		0.04		0.04
	Recovery	22	-	**Mean**	(5)	0.36	(5)	0.30	(5)	0.32
				SD		0.05		0.00		0.04
PHOS (mg/dL)	Dosing	4	---	**Mean**	(8)	6.61	(8)	6.81	(8)	6.91
				SD		0.56		0.57		0.57
		17	-	**Mean**	(9)	7.37	(9)	7.38	(8)	7.73
				SD		0.95		0.55		1.03
	Recovery	22	-	**Mean**	(5)	6.48	(5)	6.30	(5)	6.76
				SD		0.78		0.88		0.94
CA (mg/dL)	Dosing	4	---	**Mean**	(8)	9.70	(8)	9.59	(8)	9.81
				SD		0.26		0.18		0.29
		17	-	**Mean**	(9)	9.52	(9)	9.53	(8)	9.65
				SD		0.14		0.27		0.27
	Recovery	22	-	**Mean**	(5)	9.76	(5)	9.80	(5)	9.82
				SD		0.30		0.12		0.31

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	**REF**		**2**		**3**	
	Phase	**Day**	**HPD**	**Dose :**	**0 µg/day**		**30 µg/day**		**30 µg /day**	
NA (mmol/L)	Dosing	4	---	**Mean**	(8) 143.8		(8) 143.1		(8) 143.8	
				SD	0.9		1.0		1.4	
		17	-	**Mean**	(9) 143.6		(9) 143.0		(8) 143.1	
				SD	1.1		1.3		0.8	
	Recovery	22	-	**Mean**	(5) 142.2		(5) 143.2		(5) 142.8	
				SD	1.9		0.8		1.3	
K (mmol/L)	Dosing	4	---	**Mean**	(8) 3.85		(8) 4.33	†	(8) 4.39	†
				SD	0.14		0.37		0.36	
		17	-	**Mean**	(9) 4.46		(9) 4.53		(8) 4.75	
				SD	0.28		0.18		0.24	
	Recovery	22	-	**Mean**	(5) 3.84		(5) 4.00		(5) 4.00	
				SD	0.32		0.16		0.22	
CL (mmol/L)	Dosing	4	---	**Mean**	(8) 104.1		(8) 104.5		(8) 105.1	
				SD	1.4		1.8		2.0	
		17	-	**Mean**	(9) 108.0		(9) 107.7		(8) 108.1	
				SD	1.0		1.8		1.2	

Table 7

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

				Group Number:	REF		2		3	
	Phase	Day	HPD	Dose :	0 µg/day		30 µg/day		30 µg /day	
CL (mmol/L)	Recovery	22	-	Mean	(5) 106.8		(5) 106.0		(5) 106.8	
				SD	2.3		1.2		0.8	
A2M (ug/mL)	Dosing	4	---	Mean	(8) 212.1		(8) 703.8	†	(8) 887.1	†
				SD	241.1		396.4		352.9	
		17	-	Mean	(10) 33.1		(9) 521.0	†	(8) 592.0	†
				SD	49.7		260.6		243.7	
	Recovery	22	-	Mean	(5) 17.2		(5) 16.2		(5) 16.0	
				SD	8.5		5.7		4.3	
A1AGP (ug/mL)	Dosing	4	---	Mean	(8) 239.774		(8) 1906.314	†	(8) 1677.103	†
				SD	176.264		376.234		269.796	
		17	-	Mean	(10) 95.959		(9) 1491.849	†	(8) 1651.071	†
				SD	82.718		326.518		404.600	
	Recovery	22	-	Mean	(5) 62.788		(5) 47.912		(5) 57.588	
				SD	18.725		12.620		19.626	

Table 8

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
pH	pH
SG	Specific Gravity
VOLUME	Total Volume

Table 8

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Sample size is displayed in ( ) before the mean value.
SD = Standard Deviation; - = Value not applicable;
Units are displayed in the ( ) under each parameter name;
HPD = Hours Post Dose; U = Unscheduled;
e = Group mean excluded from statistics;
REF = Denotes group used as reference in the statistical test;
* = Statistically significant pairwise comparison at 0.05 level;
† = Statistically significant pairwise comparison at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level.
+ = Ascending trend sign;
- = Descending trend sign;
= Individual parameter values reported as less than or greater than limit of quantitation are set equal to the limit to calculate the average.

Table 8

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

	Phase	Day	Group Number: / Dose :	REF 0 µg/day		2 30 µg/day		3 30 µg /day	
pH (None)	Dosing	17	Mean	(10) 7.10		(10) 6.75		(10) 6.60	†
			SD	0.39		0.35		0.32	
	Recovery	22	Mean	(5) 7.30		(5) 7.20		(5) 7.00	
			SD	0.45		0.27		0.35	
SG (None)	Dosing	17	Mean	(10) 1.0322		(10) 1.0260		(10) 1.0282	
			SD	0.0205		0.0227		0.0183	
	Recovery	22	Mean	(5) 1.0556		(5) 1.0340	*	(5) 1.0440	
			SD	0.0038		0.0146		0.0234	
VOLUME (mL)	Dosing	17	Mean	(10) 14.90		(10) 17.80		(10) 11.60	
			SD	15.54		16.95		6.88	
	Recovery	22	Mean	(5) 3.70		(5) 8.20		(5) 8.00	
			SD	0.97		5.50		10.68	

Table 8

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

	Phase	Day	Group Number:	REF		2		3	
			Dose :	0 µg/day		30 µg/day		30 µg /day	
pH (None)	Dosing	17	**Mean**	(10) 6.75		(10) 6.20	†	(10) 6.20	†
			SD	0.26		0.26		0.35	
	Recovery	22	**Mean**	(5) 7.00		(5) 6.60		(5) 6.50	
			SD	0.61		0.65		0.35	
SG (None)	Dosing	17	**Mean**	(10) 1.0243		(10) 1.0288		(10) 1.0250	
			SD	0.0128		0.0164		0.0140	
	Recovery	22	**Mean**	(5) 1.0240		(5) 1.0364		(5) 1.0276	
			SD	0.0174		0.0177		0.0198	
VOLUME (mL)	Dosing	17	**Mean**	(10) 9.90		(10) 9.60		(10) 9.40	
			SD	7.03		9.05		6.98	
	Recovery	22	**Mean**	(5) 11.00		(5) 6.00		(5) 9.00	
			SD	7.38		5.09		7.52	

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

ABS = Absolute Value; OW = Organ Weight; BWT = Body Weight; BRN = Brain Weight; OW:BW = (g/g)*100; OW:BRN = g/g.
- = Value not applicable; N = Sample Size; Ratio = Group Mean / Reference Group Mean; R REF = Denotes group used as reference in the ratio calculations; SD = Standard Deviation;
REF = Denotes group used as reference in the statistical test;
e = Group mean excluded from statistics;
@ = Number examined reduced due to excluded data;
* = Statistically significant pairwise comparision at 0.05 level;
† = Statistically significant pairwise comparision at 0.01 level;
‡ = Statistically significant trend at 0.05 level;
§ = Statistically significant trend at 0.01 level;
+ = Ascending trend sign;
- = Descending trend sign;

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male - Dosing - Terminal Euthanasia

Group Number:		REF				2					3				
Dose:		0 µg/day				30 µg/day					30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD		N	Mean	Ratio	SD	
BWT	ABS	10	296 06	R REF	16 40	10	271 17	0 92	17 12	†	10	262 59	0 89	18 67	†
Brain	ABS	10	1 9061	R REF	0 0899	10	1 9159	1 01	0 1445		10	1 9082	1 00	0 0599	
	OW:BW	10	0 6449	R REF	0 0335	10	0 7087	1 10	0 0664	†	10	0 7294	1 13	0 0481	†
	OW:BRN	10	1 0000	R REF	0 0000	10	1 0000	1 00	0 0000		10	1 0000	1 00	0 0000	
Epididymis	ABS	10	1 1647	R REF	0 1713	10	1 0626	0 91	0 1281		10	1 0508	0 90	0 0665	
	OW:BW	10	0 3936	R REF	0 0536	10	0 3922	1 00	0 0428		10	0 4026	1 02	0 0442	
	OW:BRN	10	0 6112	R REF	0 0867	10	0 5570	0 91	0 0756		10	0 5512	0 90	0 0400	
Gland, Adrenal	ABS	10	0 0697	R REF	0 0068	10	0 0727	1 04	0 0149		10	0 0706	1 01	0 0107	
	OW:BW	10	0 0236	R REF	0 0021	10	0 0267	1 13	0 0045		10	0 0270	1 14	0 0044	
	OW:BRN	10	0 0366	R REF	0 0040	10	0 0383	1 04	0 0091		10	0 0371	1 01	0 0061	
Gland, Prostate	ABS	10	0 7215	R REF	0 1036	10	0 7324	1 02	0 2129		10	0 6755	0 94	0 1088	
	OW:BW	10	0 2439	R REF	0 0328	10	0 2699	1 11	0 0726		10	0 2575	1 06	0 0401	
	OW:BRN	10	0 3781	R REF	0 0476	10	0 3808	1 01	0 0941		10	0 3539	0 94	0 0556	
Heart	ABS	10	0 9152	R REF	0 0698	10	0 9242	1 01	0 1151		10	0 8795	0 96	0 1051	
	OW:BW	10	0 3097	R REF	0 0260	10	0 3405	1 10	0 0329	*	10	0 3346	1 08	0 0278	
	OW:BRN	10	0 4807	R REF	0 0388	10	0 4852	1 01	0 0758		10	0 4614	0 96	0 0583	
Kidney	ABS	10	2 1659	R REF	0 1836	10	2 2197	1 02	0 2229		10	2 0252	0 94	0 1974	
	OW:BW	10	0 7312	R REF	0 0411	10	0 8179	1 12	0 0507	†	10	0 7710	1 05	0 0495	
	OW:BRN	10	1 1356	R REF	0 0682	10	1 1600	1 02	0 0939		10	1 0607	0 93	0 0914	
Liver	ABS	10	8 3218	R REF	0 5205	10	7 7880	0 94	0 4860	*	10	7 5872	0 91	0 5920	†
	OW:BW	10	2 8131	R REF	0 1435	10	2 8771	1 02	0 1801		10	2 8905	1 03	0 1234	
	OW:BRN	10	4 3681	R REF	0 2325	10	4 0850	0 94	0 3960		10	3 9783	0 91	0 3168	*
Spleen	ABS	10	0 5951	R REF	0 0613	10	0 7700	1 29	0 1038	†	10	0 7984	1 34	0 0899	†
	OW:BW	10	0 2008	R REF	0 0147	10	0 2842	1 42	0 0352	†	10	0 3051	1 52	0 0373	†
	OW:BRN	10	0 3120	R REF	0 0264	10	0 4019	1 29	0 0431	†	10	0 4191	1 34	0 0521	†

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male - Dosing - Terminal Euthanasia

Group Number: / Dose:		REF 0 µg/day				2 30 µg/day					3 30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD		N	Mean	Ratio	SD	
Testis	ABS	10	3 2727	R REF	0 3106	10	3 4683	1 06	0 3109		10	3 2716	1 00	0 2275	
	OW:BW	10	1 1090	R REF	0 1254	10	1 2803	1 15	0 1001	*	10	1 2538	1 13	0 1447	*
	OW:BRN	10	1 7171	R REF	0 1440	10	1 8123	1 06	0 1262		10	1 7146	1 00	0 1080	
Thymus	ABS	10	0 5914	R REF	0 0676	10	0 4673	0 79	0 0934	†	10	0 4200	0 71	0 0907	†
	OW:BW	10	0 1999	R REF	0 0222	10	0 1718	0 86	0 0293	*	10	0 1591	0 80	0 0275	†
	OW:BRN	10	0 3098	R REF	0 0266	10	0 2448	0 79	0 0507	†	10	0 2199	0 71	0 0460	†

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male - Recovery - Recovery Euthanasia 1

Group Number:		REF				2					3				
Dose:		0 µg/day				30 µg/day					30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD		N	Mean	Ratio	SD	
BWT	ABS	5	331 78	R REF	39 66	5	351 50	1 06	17 44		5	334 36	1 01	13 43	
Brain	ABS	5	2 1072	R REF	0 0471	5	1 9590	0 93	0 1319	*	5	1 9408	0 92	0 0143	†
	OW:BW	5	0 6419	R REF	0 0712	5	0 5582	0 87	0 0441		5	0 5813	0 91	0 0261	
	OW:BRN	5	1 0000	R REF	0 0000	5	1 0000	1 00	0 0000		5	1 0000	1 00	0 0000	
Epididymis	ABS	5	1 3602	R REF	0 1271	5	1 2546	0 92	0 1995		5	1 4004	1 03	0 0631	
	OW:BW	5	0 4136	R REF	0 0519	5	0 3560	0 86	0 0450	*	5	0 4189	1 01	0 0134	
	OW:BRN	5	0 6457	R REF	0 0612	5	0 6387	0 99	0 0765		5	0 7216	1 12	0 0323	
Gland, Adrenal	ABS	5	0 0670	R REF	0 0112	5	0 0904	1 35	0 0185		5	0 0768	1 15	0 0144	
	OW:BW	5	0 0202	R REF	0 0026	5	0 0258	1 28	0 0058		5	0 0231	1 14	0 0050	
	OW:BRN	5	0 0318	R REF	0 0050	5	0 0458	1 44	0 0067	†	5	0 0396	1 25	0 0074	
Gland, Prostate	ABS	5	1 1332	R REF	0 2539	5	1 0192	0 90	0 1756		5	1 0446	0 92	0 1737	
	OW:BW	5	0 3431	R REF	0 0795	5	0 2913	0 85	0 0589		5	0 3129	0 91	0 0557	
	OW:BRN	5	0 5380	R REF	0 1211	5	0 5201	0 97	0 0778		5	0 5384	1 00	0 0901	
Heart	ABS	5	1 0268	R REF	0 2019	5	1 0990	1 07	0 0611		5	1 0652	1 04	0 0604	
	OW:BW	5	0 3078	R REF	0 0309	5	0 3133	1 02	0 0245		5	0 3189	1 04	0 0201	
	OW:BRN	5	0 4869	R REF	0 0925	5	0 5615	1 15	0 0143		5	0 5490	1 13	0 0342	
Kidney	ABS	5	2 4058	R REF	0 3649	5	2 3406	0 97	0 0687		5	2 2832	0 95	0 1908	
	OW:BW	5	0 7230	R REF	0 0289	5	0 6673	0 92	0 0403		5	0 6832	0 94	0 0571	
	OW:BRN	5	1 1410	R REF	0 1662	5	1 1975	1 05	0 0516		5	1 1770	1 03	0 1060	
Liver	ABS	5	8 5896	R REF	1 3878	5	8 9672	1 04	0 5403		5	8 7588	1 02	0 5137	
	OW:BW	5	2 5818	R REF	0 1539	5	2 5516	0 99	0 1092		5	2 6186	1 01	0 0777	
	OW:BRN	5	4 0739	R REF	0 6314	5	4 5995	1 13	0 4799		5	4 5142	1 11	0 2862	
Spleen	ABS	5	0 6086	R REF	0 0454	5	0 7230	1 19	0 0476	*	5	0 6604	1 09	0 1194	
	OW:BW	5	0 1856	R REF	0 0272	5	0 2057	1 11	0 0079		5	0 1980	1 07	0 0382	
	OW:BRN	5	0 2891	R REF	0 0253	5	0 3698	1 28	0 0248	*	5	0 3401	1 18	0 0606	

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male - Recovery - Recovery Euthanasia 1

Group Number: / Dose:		REF 0 µg/day				2 30 µg/day				3 30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD	N	Mean	Ratio	SD	
Testis	ABS	5	3 5472	R REF	0 2609	5	3 4582	0 97	0 3788	5	3 6938	1 04	0 2452	
	OW:BW	5	1 0751	R REF	0 0770	5	0 9839	0 92	0 0993	5	1 1070	1 03	0 0983	
	OW:BRN	5	1 6837	R REF	0 1236	5	1 7619	1 05	0 0966	5	1 9027	1 13	0 1141	*
Thymus	ABS	5	0 4938	R REF	0 0870	5	0 5536	1 12	0 0604	5	0 4270	0 86	0 0750	
	OW:BW	5	0 1515	R REF	0 0380	5	0 1572	1 04	0 0103	5	0 1284	0 85	0 0268	
	OW:BRN	5	0 2348	R REF	0 0438	5	0 2833	1 21	0 0326	5	0 2198	0 94	0 0371	

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female - Dosing - Terminal Euthanasia

Group Number:		REF				2					3				
Dose:		0 µg/day				30 µg/day					30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD		N	Mean	Ratio	SD	
BWT	ABS	10	198 73	R REF	10 80	10	194 56	0 98	10 69		10	191 82	0 97	7 14	
Brain	ABS	10	1 8610	R REF	0 0694	10	1 7868	0 96	0 0595		10	1 8407	0 99	0 0783	
	OW:BW	10	0 9383	R REF	0 0507	10	0 9203	0 98	0 0467		10	0 9604	1 02	0 0451	
	OW:BRN	10	1 0000	R REF	0 0000	10	1 0000	1 00	0 0000		10	1 0000	1 00	0 0000	
Gland, Adrenal	ABS	10	0 0882	R REF	0 0162	10	0 0886	1 00	0 0156		9@	0 0907	1 03	0 0192	
	OW:BW	10	0 0442	R REF	0 0068	10	0 0454	1 03	0 0065		9@	0 0471	1 07	0 0090	
	OW:BRN	10	0 0474	R REF	0 0088	10	0 0496	1 05	0 0085		9@	0 0490	1 03	0 0100	
Heart	ABS	10	0 7450	R REF	0 0803	10	0 7573	1 02	0 0866		10	0 7173	0 96	0 0860	
	OW:BW	10	0 3749	R REF	0 0343	10	0 3893	1 04	0 0417		10	0 3736	1 00	0 0387	
	OW:BRN	10	0 4004	R REF	0 0418	10	0 4248	1 06	0 0563		10	0 3903	0 97	0 0491	
Kidney	ABS	10	1 5273	R REF	0 0808	10	1 6343	1 07	0 0778	*	10	1 6164	1 06	0 1416	
	OW:BW	10	0 7696	R REF	0 0415	10	0 8412	1 09	0 0418	†	10	0 8417	1 09	0 0529	†
	OW:BRN	10	0 8216	R REF	0 0519	10	0 9153	1 11	0 0477	†	10	0 8787	1 07	0 0758	
Liver	ABS	10	5 4571	R REF	0 3313	10	5 6490	1 04	0 5559		10	5 8104	1 06	0 4922	
	OW:BW	10	2 7466	R REF	0 0920	10	2 9002	1 06	0 1853	*	10	3 0247	1 10	0 1541	†
	OW:BRN	10	2 9329	R REF	0 1468	10	3 1630	1 08	0 3132		10	3 1580	1 08	0 2526	
Ovary	ABS	10	0 1167	R REF	0 0158	10	0 1053	0 90	0 0180		9@	0 1113	0 95	0 0170	
	OW:BW	10	0 0588	R REF	0 0076	10	0 0542	0 92	0 0097		9@	0 0579	0 98	0 0073	
	OW:BRN	10	0 0627	R REF	0 0079	10	0 0590	0 94	0 0101		9@	0 0601	0 96	0 0085	
Spleen	ABS	10	0 4382	R REF	0 0669	10	0 6796	1 55	0 1031	†	10	0 6199	1 41	0 0555	†
	OW:BW	10	0 2202	R REF	0 0294	10	0 3492	1 59	0 0489	†	10	0 3231	1 47	0 0261	†
	OW:BRN	10	0 2353	R REF	0 0333	10	0 3803	1 62	0 0550	†	10	0 3374	1 43	0 0337	†
Thymus	ABS	10	0 4588	R REF	0 0700	10	0 3967	0 86	0 1131		10	0 3906	0 85	0 0582	
	OW:BW	10	0 2310	R REF	0 0336	10	0 2031	0 88	0 0583		10	0 2036	0 88	0 0288	
	OW:BRN	10	0 2469	R REF	0 0386	10	0 2221	0 90	0 0655		10	0 2127	0 86	0 0324	

Table 9

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female - Recovery - Recovery Euthanasia 1

Group Number: / Dose:		REF 0 µg/day				2 30 µg/day				3 30 µg /day				
		N	Mean	Ratio	SD	N	Mean	Ratio	SD	N	Mean	Ratio	SD	
BWT	ABS	5	213 64	R REF	15 61	5	213 36	1 00	6 07	5	210 36	0 98	19 63	
Brain	ABS	5	1 8336	R REF	0 1056	5	1 8732	1 02	0 1365	5	1 8450	1 01	0 0498	
	OW:BW	5	0 8609	R REF	0 0667	5	0 8788	1 02	0 0719	5	0 8846	1 03	0 1043	
	OW:BRN	5	1 0000	R REF	0 0000	5	1 0000	1 00	0 0000	5	1 0000	1 00	0 0000	
Gland, Adrenal	ABS	5	0 0866	R REF	0 0097	5	0 0904	1 04	0 0139	5	0 0900	1 04	0 0072	
	OW:BW	5	0 0407	R REF	0 0058	5	0 0424	1 04	0 0066	5	0 0431	1 06	0 0051	
	OW:BRN	5	0 0473	R REF	0 0048	5	0 0484	1 03	0 0084	5	0 0489	1 03	0 0049	
Heart	ABS	5	0 7312	R REF	0 0549	5	0 8658	1 18	0 1343	5	0 8210	1 12	0 0973	
	OW:BW	5	0 3423	R REF	0 0082	5	0 4050	1 18	0 0548	5	0 3911	1 14	0 0394	
	OW:BRN	5	0 3994	R REF	0 0297	5	0 4670	1 17	0 0981	5	0 4454	1 12	0 0555	
Kidney	ABS	5	1 5292	R REF	0 1580	5	1 6488	1 08	0 1319	5	1 7140	1 12	0 0779	
	OW:BW	5	0 7153	R REF	0 0451	5	0 7724	1 08	0 0504	5	0 8195	1 15	0 0738	*
	OW:BRN	5	0 8336	R REF	0 0651	5	0 8852	1 06	0 1104	5	0 9293	1 11	0 0428	
Liver	ABS	5	5 5626	R REF	0 4283	5	5 8068	1 04	0 3353	5	5 8276	1 05	0 5596	
	OW:BW	5	2 6032	R REF	0 0159	5	2 7204	1 05	0 1009	5	2 7738	1 07	0 1572	
	OW:BRN	5	3 0386	R REF	0 2392	5	3 1198	1 03	0 3699	5	3 1619	1 04	0 3341	
Ovary	ABS	5	0 1242	R REF	0 0347	5	0 1304	1 05	0 0384	5	0 1318	1 06	0 0253	
	OW:BW	5	0 0575	R REF	0 0121	5	0 0615	1 07	0 0196	5	0 0625	1 09	0 0088	
	OW:BRN	5	0 0674	R REF	0 0169	5	0 0695	1 03	0 0198	5	0 0717	1 06	0 0151	
Spleen	ABS	5	0 4412	R REF	0 0967	5	0 4746	1 08	0 0375	5	0 4472	1 01	0 0825	
	OW:BW	5	0 2050	R REF	0 0315	5	0 2227	1 09	0 0197	5	0 2117	1 03	0 0273	
	OW:BRN	5	0 2400	R REF	0 0471	5	0 2541	1 06	0 0241	5	0 2428	1 01	0 0468	
Thymus	ABS	5	0 4278	R REF	0 0718	5	0 4378	1 02	0 0238	5	0 3922	0 92	0 0443	
	OW:BW	5	0 2002	R REF	0 0310	5	0 2053	1 03	0 0111	5	0 1863	0 93	0 0103	
	OW:BRN	5	0 2349	R REF	0 0477	5	0 2350	1 00	0 0246	5	0 2131	0 91	0 0282	

Table 10

Summary Report of Macroscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Animals that were examined and found to be normal are not included in this report and the number of animals examined reflects the total number of animals examined grossly;
- = Value not applicable.

Table 10

Summary Report of Macroscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

	Male			Female		
Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
Animals Examined:	**10**	**10**	**10**	**10**	**10**	**10**
LIVER						
Abnormal surface	-	1	-	-	-	-
LUNG						
Abnormal color	1	1	-	-	-	-
LYMPH NODE, DRAINING						
Abnormal size	-	1	-	-	1	4
LYMPH NODE, INGUINAL						
Abnormal size	1	-	-	-	-	2
SITE, INJECTION						
Abnormal color	-	2	1	1	3	-
Abnormal consistency	-	2	2	-	4	7
SPLEEN						
Abnormal size	-	-	-	-	-	1

Table 10

Summary Report of Macroscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

	Male			Female		
Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
Animals Examined:	**5**	**5**	**5**	**5**	**5**	**5**
LYMPH NODE, DRAINING						
Abnormal size	-	1	-	-	-	1
LYMPH NODE, INGUINAL						
Abnormal size	-	-	-	-	-	1
ADIPOSE TISSUE						
Abnormal color	1	-	-	-	1	-
Abnormal consistency	1	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

NOS= Not otherwise specified; - = Value not applicable.

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
ARTERY, AORTA	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
BONE MARROW, STERNUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	0	0	10	0	0
Increased cellularity		-	10	10	-	10	10
	Minimal	-	10	10	-	10	10
BONE, STERNUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
BRAIN	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
CERVIX	Number Examined	-	-	-	10	10	10
	Unremarkable	-	-	-	10	10	10
EPIDIDYMIS	Number Examined	10	10	10	-	-	-
	Unremarkable	10	10	10	-	-	-
ESOPHAGUS	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
EYE	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	9	9	8
Mineralization, Cornea		-	-	-	-	1	-
	Minimal	-	-	-	-	1	-
Rosettes retina		-	-	-	1	-	2
	Minimal	-	-	-	1	-	2
GLAND, ADRENAL	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	9
Hypertrophy, Cortex		-	-	-	-	-	1
	Present	-	-	-	-	-	1
GLAND, HARDERIAN	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	6	9	7
Degeneration/Necrosis		-	-	-	2	-	2
	Minimal	-	-	-	2	-	2
Infiltration mononuclear cell		-	-	-	3	1	1
	Minimal	-	-	-	3	1	1
GLAND, LACRIMAL, EXTRAORBITAL	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
GLAND, MAMMARY	Number Examined	10	9	10	10	9	10
	Unremarkable	10	9	10	10	9	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
GLAND, PARATHYROID	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
GLAND, PITUITARY	Number Examined	10	10	10	10	10	10
	Unremarkable	10	9	8	9	10	8
Cyst		-	1	2	1	-	2
	Minimal	-	1	2	1	-	2
GLAND, PROSTATE	Number Examined	10	10	10	-	-	-
	Unremarkable	10	10	9	-	-	-
Infiltration mononuclear cell		-	-	1	-	-	-
	Minimal	-	-	1	-	-	-
GLAND, SALIVARY	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	9	10	10
Hypertrophy		-	-	-	1	-	-
	Minimal	-	-	-	1	-	-
GLAND, SEMINAL VESICLE	Number Examined	10	10	10	-	-	-
	Unremarkable	10	10	10	-	-	-
GLAND, THYROID	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
GUT-ASSOCIATED LYMPHOID TISSUE	Number Examined	10	10	10	8	10	10
	Unremarkable	10	10	10	8	9	10
Mineralization, Germinal center		-	-	-	-	1	-
	Minimal	-	-	-	-	1	-
HEART	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
JOINT	Number Examined	10	10	10	10	10	10
	Unremarkable	10	7	10	9	8	7
Inflammation, Extra-capsular		-	3	-	-	2	3
	Minimal	-	3	-	-	2	3
Physeal dysplasia		-	-	-	1	-	-
	Minimal	-	-	-	1	-	-
KIDNEY	Number Examined	10	10	10	10	10	10
	Unremarkable	9	9	9	8	6	10
Tubular basophilia		-	1	-	-	1	-
	Minimal	-	1	-	-	1	-
Infiltration mononuclear cell		-	-	1	2	3	-
	Minimal	-	-	1	2	3	-
Dilatation, Pelvis		1	-	-	-	-	-
	Minimal	1	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
LARGE INTESTINE, CECUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
LARGE INTESTINE, COLON	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	9
Infiltration mixed cell, Mucosa		-	-	-	-	-	1
	Minimal	-	-	-	-	-	1
LIVER	Number Examined	10	10	10	10	10	10
	Unremarkable	10	5	3	10	0	3
Vacuolation, Hepatocyte; Periportal		-	5	7	-	10	7
	Minimal	-	5	7	-	10	7
LUNG	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	9	9	10
Infiltration mixed cell		-	-	-	1	1	-
	Minimal	-	-	-	1	1	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
LYMPH NODE, DRAINING	Number Examined	10	9	10	10	10	10
	Unremarkable	8	1	1	8	1	1
Increased cellularity, Plasma cell		-	7	8	-	9	7
	Minimal	-	1	4	-	1	1
	Mild	-	4	3	-	1	5
	Moderate	-	2	1	-	7	1
Increased cellularity, Germinal center		2	6	8	2	5	6
	Minimal	1	2	2	1	3	4
	Mild	1	4	6	1	2	2
LYMPH NODE, INGUINAL	Number Examined	9	10	10	10	10	10
	Unremarkable	8	5	4	9	4	1
Increased cellularity, Germinal center		1	5	6	1	6	9
	Minimal	-	1	1	1	3	6
	Mild	1	4	5	-	3	3
Increased cellularity, Plasma cell		-	1	1	-	2	4
	Minimal	-	1	1	-	2	4
LYMPH NODE, MESENTERIC	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
MUSCLE, SKELETAL	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
NERVE, OPTIC	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
NERVE, PERIPHERAL	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
OVARY	Number Examined	-	-	-	10	10	10
	Unremarkable	-	-	-	10	10	10
OVIDUCT	Number Examined	-	-	-	10	10	10
	Unremarkable	-	-	-	10	10	10
PANCREAS	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	6	10
Atrophy, Acinar cell		-	-	-	-	4	-
	Minimal	-	-	-	-	4	-
Infiltration mononuclear cell, Interstitium		-	-	-	-	1	-
	Minimal	-	-	-	-	1	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
SITE, INJECTION	Number Examined	10	10	10	10	10	10
	Unremarkable	6	0	0	5	0	0
Inflammation		4	10	10	5	10	10
	Minimal	4	-	-	5	-	-
	Mild	-	7	5	-	7	9
	Moderate	-	3	5	-	3	1
Edema		-	9	9	-	10	10
	Mild	-	8	8	-	9	9
	Moderate	-	1	1	-	1	1
SKIN	Number Examined	10	10	10	10	9	10
	Unremarkable	10	10	10	10	9	10
SMALL INTESTINE, DUODENUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
SMALL INTESTINE, ILEUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
SMALL INTESTINE, JEJUNUM	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
SPINAL CORD	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
SPLEEN	Number Examined	10	10	10	10	10	10
	Unremarkable	10	0	0	10	0	0
Increased cellularity, Germinal center		-	5	5	-	6	5
	Minimal	-	5	5	-	6	5
Increased cellularity, Hematopoietic cell		-	10	10	-	9	10
	Minimal	-	10	10	-	9	10
STOMACH	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	9	10	9	10
Infiltration mononuclear cell, Serosa		-	-	-	-	1	-
	Minimal	-	-	-	-	1	-
Erosion		-	-	1	-	-	-
	Minimal	-	-	1	-	-	-
TESTIS	Number Examined	10	10	10	-	-	-
	Unremarkable	10	10	10	-	-	-
THYMUS	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
TONGUE	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Dosing, Necropsy Status: Terminal Euthanasia

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**10**	**10**	**10**	**10**	**10**	**10**
TRACHEA	Number Examined	10	10	10	10	10	10
	Unremarkable	10	10	10	10	10	10
URETER	Number Examined	10	9	10	10	10	10
	Unremarkable	10	9	10	10	10	10
URINARY BLADDER	Number Examined	10	10	10	9	10	10
	Unremarkable	10	10	10	9	10	10
UTERUS	Number Examined	-	-	-	10	10	10
	Unremarkable	-	-	-	10	10	10
VAGINA	Number Examined	-	-	-	10	10	10
	Unremarkable	-	-	-	10	10	10
ADIPOSE TISSUE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
ARTERY, AORTA	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
BONE MARROW, STERNUM	Number Examined	5	5	5	5	5	5
	Unremarkable	5	5	5	5	5	5
BONE, STERNUM	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
BRAIN	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
CERVIX	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
EPIDIDYMIS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
ESOPHAGUS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
EYE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
GLAND, ADRENAL	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, HARDERIAN	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, LACRIMAL, EXTRAORBITAL	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, MAMMARY	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, PARATHYROID	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, PITUITARY	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, PROSTATE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, SALIVARY	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
GLAND, SEMINAL VESICLE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GLAND, THYROID	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
GUT-ASSOCIATED LYMPHOID TISSUE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
HEART	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
JOINT	Number Examined	5	5	5	5	5	5
	Unremarkable	5	5	5	5	4	4
Physeal dysplasia		-	-	-	-	1	1
	Minimal	-	-	-	-	1	1
KIDNEY	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
LARGE INTESTINE, CECUM	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
LARGE INTESTINE, COLON	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
LIVER	Number Examined	5	5	5	5	5	5
	Unremarkable	5	5	5	5	5	5
LUNG	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
LYMPH NODE, DRAINING	Number Examined	4	5	5	5	5	5
	Unremarkable	4	0	0	4	0	0
Increased cellularity, Plasma cell		-	4	5	-	4	3
	Minimal	-	4	5	-	4	3
Increased cellularity, Germinal center		-	4	4	1	3	5
	Minimal	-	3	2	1	2	4
	Mild	-	1	2	-	1	1
Infiltration, Macrophage		-	3	4	-	3	4
	Minimal	-	2	2	-	1	1
	Mild	-	1	2	-	2	3

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
LYMPH NODE, INGUINAL	Number Examined	5	5	5	5	5	5
	Unremarkable	3	2	3	3	4	2
Increased cellularity, Germinal center		2	3	2	2	1	3
	Minimal	2	3	2	2	1	3
Increased cellularity, Plasma cell		-	-	-	-	-	1
	Minimal	-	-	-	-	-	1
Infiltration, Macrophage		-	-	1	-	-	1
	Minimal	-	-	1	-	-	1
LYMPH NODE, MESENTERIC	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
MUSCLE, SKELETAL	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
NERVE, OPTIC	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
NERVE, PERIPHERAL	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
OVARY	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
OVIDUCT	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
PANCREAS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
SITE, INJECTION	Number Examined	5	5	5	5	5	5
	Unremarkable	5	0	0	5	0	0
Inflammation		-	5	5	-	5	5
	Minimal	-	5	5	-	5	5
SKIN	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
SMALL INTESTINE, DUODENUM	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
SMALL INTESTINE, ILEUM	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
SMALL INTESTINE, JEJUNUM	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
SPINAL CORD	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
SPLEEN	Number Examined	5	5	5	5	5	5
	Unremarkable	5	4	4	5	3	3
Increased cellularity, Germinal center		-	1	1	-	2	2
	Minimal	-	1	1	-	2	2
STOMACH	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
TESTIS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
THYMUS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
TONGUE	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
TRACHEA	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
URETER	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
URINARY BLADDER	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-

Table 11

Summary Report of Microscopic Observations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study Phase: Recovery, Necropsy Status: Recovery Euthanasia 1

		Male			Female		
	Group Number:	**1**	**2**	**3**	**1**	**2**	**3**
	Dose:	**0 µg/day**	**30 µg/day**	**30 µg /day**	**0 µg/day**	**30 µg/day**	**30 µg /day**
	No. Animals Per Dose Group:	**5**	**5**	**5**	**5**	**5**	**5**
UTERUS	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
VAGINA	Number Examined	-	-	-	-	-	-
	Unremarkable	-	-	-	-	-	-
ADIPOSE TISSUE	Number Examined	1	-	-	-	1	-
	Unremarkable	0	-	-	-	0	-
Inflammation		1	-	-	-	-	-
	Mild	1	-	-	-	-	-
Fibrosis		1	-	-	-	-	-
	Minimal	1	-	-	-	-	-
Infiltration mononuclear cell		-	-	-	-	1	-
	Mild	-	-	-	-	1	-

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Parameter	Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Edema - Left	Dosing	1	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.63	0.51	0.001 **
			3: BNT162b3c	15	0.80	0.55	0.001 **
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.19	0.51	0.001 **
			3: BNT162b3c	15	1.43	0.12	0.001 **
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.33	0.45	0.001 **
			3: BNT162b3c	15	1.54	0.46	0.001 **

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Parameter	Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Erythema - Left	Dosing	1	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.03	0.13	0.682
			3: BNT162b3c	15	0.04	0.13	0.270
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.23	0.27	0.001 **
			3: BNT162b3c	15	0.41	0.17	0.001 **
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.00	0.00	0.999
			3: BNT162b3c	15	0.09	0.20	0.050 *

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Parameter	Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Edema - Left	Dosing	1	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.28	0.57	0.001 **
			3: BNT162b3c	15	1.08	0.58	0.001 **
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.44	0.23	0.001 **
			3: BNT162b3c	15	1.47	0.28	0.001 **
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.64	0.34	0.001 **
			3: BNT162b3c	15	1.78	0.27	0.001 **

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Parameter	Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Erythema - Left	Dosing	1	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.56	0.38	0.001 **
			3: BNT162b3c	15	0.66	0.17	0.001 **
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.50	0.09	0.001 **
			3: BNT162b3c	15	0.58	0.11	0.001 **
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.33	0.22	0.001 **
			3: BNT162b3c	15	0.60	0.14	0.001 **

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Parameter	Phase	Group	N	Mean	Standard Deviation
Edema - Left	Recovery	2: BNT162b2 (V9)	4	1.08	0.17
		3: BNT162b3c	5	0.80	0.18
Erythema - Left	Recovery	2: BNT162b2 (V9)	4	0.00	0.00
		3: BNT162b3c	5	0.00	0.00

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Injection Site Score

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Parameter	Phase	Group	N	Mean	Standard Deviation
Edema - Left	Recovery	2: BNT162b2 (V9)	5	1.07	0.15
		3: BNT162b3c	5	1.13	0.18
Erythema - Left	Recovery	2: BNT162b2 (V9)	5	0.13	0.18
		3: BNT162b3c	5	0.33	0.24

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Body Temperature (Deg C)

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Dosing	1	1: Saline	15	38.31	0.35	REF
		2: BNT162b2 (V9)	15	38.85	0.36	0.001 **
		3: BNT162b3c	15	39.02	0.40	0.001 **
Dosing	8	1: Saline	15	37.07	0.37	REF
		2: BNT162b2 (V9)	15	38.05	0.62	0.001 **
		3: BNT162b3c	15	38.33	0.43	0.001 **
Dosing	15	1: Saline	15	37.34	0.35	REF
		2: BNT162b2 (V9)	15	38.37	0.42	0.001 **
		3: BNT162b3c	15	38.43	0.36	0.001 **

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Body Temperature (Deg C)

20GR142: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Phase	Day	Group	N	Mean	Standard Deviation	Pairwise p-value
Dosing	1	1: Saline	15	38.08	0.44	REF
		2: BNT162b2 (V9)	15	38.50	0.53	0.044 *
		3: BNT162b3c	15	38.58	0.36	0.009 **
Dosing	8	1: Saline	15	37.81	0.38	REF
		2: BNT162b2 (V9)	15	38.47	0.44	0.001 **
		3: BNT162b3c	15	38.73	0.40	0.001 **
Dosing	15	1: Saline	15	38.02	0.74	REF
		2: BNT162b2 (V9)	15	38.15	0.54	0.963
		3: BNT162b3c	15	38.35	0.31	0.174

REF: Denotes group used as reference in the statistical tests.
* Statistically significant at 0.05 level ** Statistically significant at 0.01 level

Dead Animal Status Report

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Pfizer

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
001	M	1	1677	22 Jul 2020 08:53:06 AM	Dosing	17	22 Jul 2020 08:53:07 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
002	M	1	1787	22 Jul 2020 08:50:04 AM	Dosing	17	22 Jul 2020 08:50:05 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
003	M	1	727	22 Jul 2020 08:57:39 AM	Dosing	17	22 Jul 2020 08:57:40 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
004	M	1	598	22 Jul 2020 09:02:59 AM	Dosing	17	22 Jul 2020 09:02:59 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
005	M	1	727	22 Jul 2020 09:37:05 AM	Dosing	17	22 Jul 2020 09:37:06 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
006	M	1	1687	22 Jul 2020 09:46:22 AM	Dosing	17	22 Jul 2020 09:46:22 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
007	M	1	807	22 Jul 2020 09:58:31 AM	Dosing	17	22 Jul 2020 09:58:32 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
008	M	1	727	22 Jul 2020 10:17:27 AM	Dosing	17	22 Jul 2020 10:17:28 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
009	M	1	808	22 Jul 2020 10:30:03 AM	Dosing	17	22 Jul 2020 10:30:04 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
010	M	1	1777	22 Jul 2020 10:41:36 AM	Dosing	17	22 Jul 2020 10:41:36 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
011	M	1	1057	13 Aug 2020 07:32:04 AM	Recovery	22	13 Aug 2020 07:32:05 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			

(b) (6)

Dead Animal Status Report

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Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
012	M	1	232	13 Aug 2020 08:15:17 AM	Recovery	22	13 Aug 2020 08:15:18 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
013	M	1	1307	13 Aug 2020 08:29:36 AM	Recovery	22	13 Aug 2020 08:29:37 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
014	M	1	1777	13 Aug 2020 09:02:02 AM	Recovery	22	13 Aug 2020 09:02:03 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
015	M	1	232	13 Aug 2020 09:23:27 AM	Recovery	22	13 Aug 2020 09:23:28 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
016	M	2	1057	22 Jul 2020 08:42:46 AM	Dosing	17	22 Jul 2020 08:42:47 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
017	M	2	1777	22 Jul 2020 08:48:01 AM	Dosing	17	22 Jul 2020 08:48:01 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
018	M	2	1307	22 Jul 2020 09:01:59 AM	Dosing	17	22 Jul 2020 09:01:59 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
019	M	2	807	22 Jul 2020 09:08:39 AM	Dosing	17	22 Jul 2020 09:08:39 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
020	M	2	1677	22 Jul 2020 09:41:57 AM	Dosing	17	22 Jul 2020 09:41:57 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
021	M	2	1308	22 Jul 2020 09:53:57 AM	Dosing	17	22 Jul 2020 09:53:58 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
022	M	2	598	22 Jul 2020 09:59:56 AM	Dosing	17	22 Jul 2020 09:59:56 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			

(b) (6)

Dead Animal Status Report

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Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
023	M	2	1787	22 Jul 2020 10:29:38 AM	Dosing	17	22 Jul 2020 10:29:38 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
024	M	2	1307	22 Jul 2020 10:38:09 AM	Dosing	17	22 Jul 2020 10:38:10 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
025	M	2	807	22 Jul 2020 10:44:39 AM	Dosing	17	22 Jul 2020 10:44:40 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
026	M	2	1777	13 Aug 2020 07:36:45 AM	Recovery	22	13 Aug 2020 07:36:46 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
027	M	2	598	13 Aug 2020 08:25:28 AM	Recovery	22	13 Aug 2020 08:25:29 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
028	M	2	807	13 Aug 2020 08:44:11 AM	Recovery	22	13 Aug 2020 08:44:12 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
029	M	2	1057	13 Aug 2020 09:12:59 AM	Recovery	22	13 Aug 2020 09:13:00 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
030	M	2	598	13 Aug 2020 09:25:22 AM	Recovery	22	13 Aug 2020 09:25:24 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
031	M	3	1308	22 Jul 2020 08:47:19 AM	Dosing	17	22 Jul 2020 08:47:19 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
032	M	3	1687	22 Jul 2020 08:55:08 AM	Dosing	17	22 Jul 2020 08:55:08 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
033	M	3	808	22 Jul 2020 09:03:00 AM	Dosing	17	22 Jul 2020 09:03:01 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			

(b) (6)

Dead Animal Status Report

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
034	M	3	1057	22 Jul 2020 09:25:56 AM	Dosing	17	22 Jul 2020 09:25:56 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
035	M	3	808	22 Jul 2020 09:45:48 AM	Dosing	17	22 Jul 2020 09:45:49 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
036	M	3	1307	22 Jul 2020 09:56:54 AM	Dosing	17	22 Jul 2020 09:56:55 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
037	M	3	1057	22 Jul 2020 10:14:20 AM	Dosing	17	22 Jul 2020 10:14:21 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
038	M	3	1677	22 Jul 2020 10:27:35 AM	Dosing	17	22 Jul 2020 10:27:36 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
039	M	3	1687	22 Jul 2020 10:38:42 AM	Dosing	17	22 Jul 2020 10:38:43 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
040	M	3	598	22 Jul 2020 10:52:34 AM	Dosing	17	22 Jul 2020 10:52:35 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
041	M	3	808	13 Aug 2020 08:12:59 AM	Recovery	22	13 Aug 2020 08:13:00 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
042	M	3	1057	13 Aug 2020 08:26:36 AM	Recovery	22	13 Aug 2020 08:26:36 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
043	M	3	808	13 Aug 2020 08:59:12 AM	Recovery	22	13 Aug 2020 08:59:13 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
044	M	3	1307	13 Aug 2020 09:10:48 AM	Recovery	22	13 Aug 2020 09:10:49 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			

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Dead Animal Status Report

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
045	M	3	807	13 Aug 2020 09:30:28 AM	Recovery	22	13 Aug 2020 09:30:29 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
046	F	1	727	22 Jul 2020 10:55:54 AM	Dosing	17	22 Jul 2020 10:55:55 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
047	F	1	808	22 Jul 2020 11:13:53 AM	Dosing	17	22 Jul 2020 11:13:53 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
048	F	1	1307	22 Jul 2020 11:32:53 AM	Dosing	17	22 Jul 2020 11:32:54 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
049	F	1	1777	22 Jul 2020 11:53:48 AM	Dosing	17	22 Jul 2020 11:53:48 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
050	F	1	1307	22 Jul 2020 12:09:56 PM	Dosing	17	22 Jul 2020 12:09:57 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
051	F	1	807	22 Jul 2020 12:17:24 PM	Dosing	17	22 Jul 2020 12:17:24 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
052	F	1	1677	22 Jul 2020 12:29:39 PM	Dosing	17	22 Jul 2020 12:29:39 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
053	F	1	1057	22 Jul 2020 12:41:24 PM	Dosing	17	22 Jul 2020 12:41:24 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
054	F	1	1308	22 Jul 2020 01:06:59 PM	Dosing	17	22 Jul 2020 01:06:59 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
055	F	1	1677	22 Jul 2020 01:13:05 PM	Dosing	17	22 Jul 2020 01:13:06 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			

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Dead Animal Status Report

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
056	F	1	808	13 Aug 2020 09:40:42 AM	Recovery	22	13 Aug 2020 09:40:43 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
057	F	1	1777	13 Aug 2020 10:06:54 AM	Recovery	22	13 Aug 2020 10:06:55 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
058	F	1	808	13 Aug 2020 10:24:39 AM	Recovery	22	13 Aug 2020 10:24:40 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
059	F	1	1307	13 Aug 2020 10:37:40 AM	Recovery	22	13 Aug 2020 10:37:41 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
060	F	1	598	13 Aug 2020 11:11:42 AM	Recovery	22	13 Aug 2020 11:11:43 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
061	F	2	1057	22 Jul 2020 10:59:29 AM	Dosing	17	22 Jul 2020 10:59:30 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
062	F	2	1687	22 Jul 2020 11:28:12 AM	Dosing	17	22 Jul 2020 11:28:13 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
063	F	2	807	22 Jul 2020 11:34:48 AM	Dosing	17	22 Jul 2020 11:34:48 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
064	F	2	808	22 Jul 2020 12:02:00 PM	Dosing	17	22 Jul 2020 12:02:01 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
065	F	2	1308	22 Jul 2020 12:10:34 PM	Dosing	17	22 Jul 2020 12:10:35 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
066	F	2	598	22 Jul 2020 12:26:23 PM	Dosing	17	22 Jul 2020 12:26:24 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			

(b) (6)

Dead Animal Status Report

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
067	F	2	808	22 Jul 2020 12:39:45 PM	Dosing	17	22 Jul 2020 12:39:46 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
068	F	2	1307	22 Jul 2020 12:48:19 PM	Dosing	17	22 Jul 2020 12:48:20 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
069	F	2	1687	22 Jul 2020 01:10:04 PM	Dosing	17	22 Jul 2020 01:10:05 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
070	F	2	598	22 Jul 2020 01:20:52 PM	Dosing	17	22 Jul 2020 01:20:53 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
071	F	2	1307	13 Aug 2020 09:45:17 AM	Recovery	22	13 Aug 2020 09:45:18 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
072	F	2	807	13 Aug 2020 10:10:49 AM	Recovery	22	13 Aug 2020 10:10:51 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
073	F	2	232	13 Aug 2020 10:31:30 AM	Recovery	22	13 Aug 2020 10:31:32 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
074	F	2	807	13 Aug 2020 10:57:42 AM	Recovery	22	13 Aug 2020 10:57:44 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
075	F	2	1057	13 Aug 2020 11:14:34 AM	Recovery	22	13 Aug 2020 11:14:35 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
076	F	3	1308	22 Jul 2020 11:01:58 AM	Dosing	17	22 Jul 2020 11:01:59 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
077	F	3	727	22 Jul 2020 11:34:04 AM	Dosing	17	22 Jul 2020 11:34:04 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			

(b) (6)

Dead Animal Status Report

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
078	F	3	1057	22 Jul 2020 11:39:37 AM	Dosing	17	22 Jul 2020 11:39:38 AM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
079	F	3	727	22 Jul 2020 12:04:24 PM	Dosing	17	22 Jul 2020 12:04:26 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
080	F	3	1787	22 Jul 2020 12:15:12 PM	Dosing	17	22 Jul 2020 12:15:13 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
081	F	3	1687	22 Jul 2020 12:23:21 PM	Dosing	17	22 Jul 2020 12:23:22 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
082	F	3	727	22 Jul 2020 12:40:34 PM	Dosing	17	22 Jul 2020 12:40:35 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
083	F	3	807	22 Jul 2020 01:00:12 PM	Dosing	17	22 Jul 2020 01:00:12 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
084	F	3	1777	22 Jul 2020 01:15:45 PM	Dosing	17	22 Jul 2020 01:15:45 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
085	F	3	727	22 Jul 2020 01:18:09 PM	Dosing	17	22 Jul 2020 01:18:10 PM	Y
Death Status: Terminal Euthanasia					**Death Type:** Scheduled			
086	F	3	1057	13 Aug 2020 09:54:23 AM	Recovery	22	13 Aug 2020 09:54:24 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
087	F	3	598	13 Aug 2020 10:20:29 AM	Recovery	22	13 Aug 2020 10:20:29 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
088	F	3	1057	13 Aug 2020 10:34:13 AM	Recovery	22	13 Aug 2020 10:34:14 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			

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Dead Animal Status Report

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Animal #	Sex	Group	User #	Date/Time Entered	Phase Name	Day of Phase	Date/Time of Death	Approx
089	F	3	1777	13 Aug 2020 11:07:56 AM	Recovery	22	13 Aug 2020 11:07:57 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
090	F	3	808	13 Aug 2020 11:19:57 AM	Recovery	22	13 Aug 2020 11:19:58 AM	Y
Death Status: Recovery Euthanasia 1					**Death Type:** Scheduled			
P-054#	F	-	1077	01 Jul 2020 01:57:34 PM	PID	8	01 Jul 2020 01:57:15 PM	Y
Death Status: Found Dead					**Death Type:** Unscheduled Death			
Comment: Died after blood collection								

= Pretest

(b) (6)

Dead Animal Status Report Audit Trail

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Pristima® Version 7.4.3 Build 25

Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

No Audit Trail

Appendix 2

Clinical Signs - Daily

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Animals were considered normal (data not displayed in table) unless indicated otherwise.
Note: Each interval will be concatenated with the phase name abbreviation.

- Value not applicable

Day(s) Observed - PID = Prior to Initiation of Dosing, D = Dosing, R = Recovery

Appendix 2

Clinical Signs - Daily

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
1	0 µg/day	012	Thin Appearance		D8	1
2	30 µg/day	024	Hair Loss	Abdomen, Thinning	D8, 15	2
		029	Tail Crooked		PID1-12, D1-17, R1-22	51

Appendix 2

Clinical Signs - Daily

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
2	30 µg/day	067	Hair Loss	Forelimb, Bilateral, Thinning	D15	1
3	30 µg /day	083	Lesion	Lumbar, Dorsal	D1	1

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Note: Animals were considered normal (data not displayed in table) unless indicated otherwise.
Note: Each interval will be concatenated with the phase name abbreviation.

- Value not applicable

Day(s) Observed - PID = Prior to Initiation of Dosing, D = Dosing, R = Recovery

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
1	0 µg/day	001	No Ocular Abnormality	Bilateral	PID7, D15	2
		002	No Ocular Abnormality	Bilateral	PID7, D15	2
		003	No Ocular Abnormality	Bilateral	PID7, D15	2
		004	No Ocular Abnormality	Bilateral	PID7, D15	2
		005	No Ocular Abnormality	Bilateral	PID7, D15	2
		006	No Ocular Abnormality	Bilateral	PID7, D15	2
		007	No Ocular Abnormality	Bilateral	PID7, D15	2
		008	Vitreous, Hemorrhage	Mild, Left, Temporal, Ventral	PID7, D15	2
		009	No Ocular Abnormality	Bilateral	PID7, D15	2
		010	Retina, Tortuous Vessels	Minimal, Left, Generalized, Multifocal	PID7, D15	2
		011	Vitreous, Hyaloid Remnant	Minimal, Right, Central, Central	PID7, D15	2
		012	No Ocular Abnormality	Bilateral	PID7, D15	2
		013	No Ocular Abnormality	Bilateral	PID7, D15	2
		014	Keratic Precipitates	Mild, Left, Equatorial	PID7, D15	2
		015	No Ocular Abnormality	Bilateral	PID7, D15	2
2	30 µg/day	016	No Ocular Abnormality	Bilateral	PID7, D15	2
		017	No Ocular Abnormality	Bilateral	PID7, D15	2
		018	No Ocular Abnormality	Bilateral	PID7, D15	2

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
2	30 µg/day	019	No Ocular Abnormality	Bilateral	PID7, D15	2
		020	No Ocular Abnormality	Bilateral	PID7, D15	2
		021	No Ocular Abnormality	Bilateral	PID7, D15	2
		022	No Ocular Abnormality	Bilateral	PID7, D15	2
		023	No Ocular Abnormality	Bilateral	PID7, D15	2
		024	No Ocular Abnormality	Bilateral	PID7, D15	2
		025	No Ocular Abnormality	Bilateral	PID7, D15	2
		026	No Ocular Abnormality	Bilateral	PID7, D15	2
		027	No Ocular Abnormality	Bilateral	PID7, D15	2
		028	No Ocular Abnormality	Bilateral	PID7, D15	2
		029	No Ocular Abnormality	Bilateral	PID7, D15	2
		030	No Ocular Abnormality	Bilateral	PID7, D15	2
3	30 µg /day	031	No Ocular Abnormality	Bilateral	PID7, D15	2
		032	No Ocular Abnormality	Bilateral	PID7, D15	2
		033	No Ocular Abnormality	Bilateral	PID7, D15	2
		034	No Ocular Abnormality	Bilateral	PID7, D15	2
		035	No Ocular Abnormality	Bilateral	PID7, D15	2
		036	No Ocular Abnormality	Bilateral	PID7, D15	2
		037	No Ocular Abnormality	Bilateral	PID7, D15	2

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
3	30 µg /day	038	No Ocular Abnormality	Bilateral	PID7, D15	2
		039	No Ocular Abnormality	Bilateral	PID7, D15	2
		040	No Ocular Abnormality	Bilateral	PID7, D15	2
		041	Keratic Precipitates	Mild, Right, Equatorial	PID7, D15	2
		042	No Ocular Abnormality	Bilateral	PID7, D15	2
		043	No Ocular Abnormality	Bilateral	PID7, D15	2
		044	No Ocular Abnormality	Bilateral	PID7, D15	2
		045	No Ocular Abnormality	Bilateral	PID7, D15	2

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
1	0 µg/day	046	No Ocular Abnormality	Bilateral	PID8, D16	2
		047	No Ocular Abnormality	Bilateral	PID8, D16	2
		048	No Ocular Abnormality	Bilateral	PID8, D16	2
		049	Keratic Precipitates	Mild, Left, Equatorial	D16	1
			No Ocular Abnormality	Bilateral	PID8	1
		050	No Ocular Abnormality	Bilateral	PID8, D16	2
		051	No Ocular Abnormality	Bilateral	PID8, D16	2
		052	No Ocular Abnormality	Bilateral	PID8, D16	2
		053	No Ocular Abnormality	Bilateral	PID8, D16	2
		054	No Ocular Abnormality	Bilateral	PID8, D16	2
		055	No Ocular Abnormality	Bilateral	PID8, D16	2
		056	No Ocular Abnormality	Bilateral	PID8, D16	2
		057	No Ocular Abnormality	Bilateral	PID8, D16	2
		058	No Ocular Abnormality	Bilateral	PID8, D16	2
		059	No Ocular Abnormality	Bilateral	PID8, D16	2
		060	No Ocular Abnormality	Bilateral	PID8, D16	2
2	30 µg/day	061	No Ocular Abnormality	Bilateral	PID8, D16	2
		062	No Ocular Abnormality	Bilateral	PID8, D16	2
		063	No Ocular Abnormality	Bilateral	PID8, D16	2

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
2	30 µg/day	064	No Ocular Abnormality	Bilateral	PID8, D16	2
		065	No Ocular Abnormality	Bilateral	PID8, D16	2
		066	No Ocular Abnormality	Bilateral	PID8, D16	2
		067	No Ocular Abnormality	Bilateral	PID8, D16	2
		068	No Ocular Abnormality	Bilateral	PID8, D16	2
		069	No Ocular Abnormality	Bilateral	PID8, D16	2
		070	No Ocular Abnormality	Bilateral	PID8, D16	2
		071	No Ocular Abnormality	Bilateral	PID8, D16	2
		072	No Ocular Abnormality	Bilateral	PID8, D16	2
		073	No Ocular Abnormality	Bilateral	PID8, D16	2
		074	No Ocular Abnormality	Bilateral	PID8, D16	2
		075	No Ocular Abnormality	Bilateral	PID8, D16	2
3	30 µg /day	076	No Ocular Abnormality	Bilateral	PID8, D16	2
		077	No Ocular Abnormality	Bilateral	PID8, D16	2
		078	No Ocular Abnormality	Bilateral	PID8, D16	2
		079	No Ocular Abnormality	Bilateral	PID8, D16	2
		080	No Ocular Abnormality	Bilateral	PID8, D16	2
		081	No Ocular Abnormality	Bilateral	PID8, D16	2
		082	No Ocular Abnormality	Bilateral	PID8, D16	2

Appendix 3

Ocular Exam

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Observations	Modifier	Day(s) Observed	Total Days Seen
3	30 µg /day	083	No Ocular Abnormality	Bilateral	PID8, D16	2
		084	No Ocular Abnormality	Bilateral	PID8, D16	2
		085	No Ocular Abnormality	Bilateral	PID8, D16	2
		086	No Ocular Abnormality	Bilateral	PID8, D16	2
		087	No Ocular Abnormality	Bilateral	PID8, D16	2
		088	No Ocular Abnormality	Bilateral	PID9, D16	2
		089	No Ocular Abnormality	Bilateral	PID8, D16	2
		090	No Ocular Abnormality	Bilateral	PID8, D16	2

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Grp Num = Group Number; Animal Num= Animal Number; - = Value not applicable; NW = Not Weighed; e = Excluded.
PID = Prior to the Initiation of Dosing.

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Day:	PID		Dosing					Recovery
				1	6	1	4	8	11	15	1
1	0 µg/day	001		178.3	217.8	252.9	239.1	270.5	284.3	290.1	-
		002		198.5	242.8	283.8	271.6	307.6	321.9	339.6	-
		003		180.0	226.4	270.2	259.1	291.0	306.8	318.6	-
		004		183.2	217.4	265.1	258.3	303.8	312.2	333.9	-
		005		185.6	223.1	263.8	254.0	286.4	301.4	315.8	-
		006		179.9	221.6	266.5	256.3	293.7	305.5	321.7	-
		007		195.6	232.1	270.8	259.9	295.6	310.2	321.8	-
		008		201.9	233.3	271.0	257.5	289.2	298.7	311.9	-
		009		178.5	210.0	248.2	240.6	267.4	276.0	293.1	-
		010		185.8	225.4	262.5	248.3	281.7	292.1	309.1	-
		011		195.6	227.8	264.1	257.1	291.2	296.8	310.2	313.8
		012		200.1	239.3	280.0	245.9	200.9	264.6	296.6	307.4
		013		183.6	213.9	243.1	233.1	262.7	268.5	283.4	287.6
		014		178.6	214.6	251.1	237.2	268.9	281.5	290.2	288.9
		015		190.5	233.7	278.9	264.4	298.4	317.0	336.1	340.8
2	30 µg/day	016		199.1	231.5	262.6	237.4	274.1	270.5	289.2	-
		017		182.0	218.3	266.9	249.5	291.2	292.1	307.0	-
		018		186.8	227.1	267.1	253.9	283.3	283.7	298.2	-
		019		193.4	233.0	269.3	258.6	287.4	292.6	309.8	-
		020		178.1	216.7	253.1	230.2	265.7	261.6	276.3	-
		021		185.4	228.6	269.4	248.9	279.2	276.5	296.0	-
		022		193.3	235.0	265.8	243.9	276.6	276.2	298.3	-
		023		184.6	222.6	259.9	241.4	273.0	281.2	296.1	-
		024		175.1	213.4	257.4	234.4	267.7	261.0	275.4	-
		025		200.1	239.9	284.6	268.0	311.6	307.9	325.5	-
		026		192.2	231.0	274.9	249.7	293.8	298.6	317.7	310.9
		027		186.8	222.2	265.8	243.4	286.4	284.0	306.3	306.0
		028		194.4	224.6	262.6	244.2	276.7	275.1	299.5	290.0
		029		195.7	239.4	280.0	259.6	295.1	297.4	320.4	312.8

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Day:	PID		Dosing					Recovery
				1	6	1	4	8	11	15	1
2	30 µg/day	030		179.2	215.5	268.3	251.0	292.4	297.3	322.3	322.8
3	30 µg /day	031		191.0	216.0	244.2	224.5	250.4	250.3	268.7	-
		032		178.1	210.7	254.6	234.7	264.1	258.3	274.4	-
		033		194.1	236.2	291.6	269.8	310.8	315.8	330.5	-
		034		187.5	222.4	259.6	233.8	267.7	264.5	280.4	-
		035		188.1	227.2	256.5	227.2	259.4	245.6	267.8	-
		036		195.0	239.8	274.1	254.1	288.9	287.8	308.0	-
		037		192.8	235.5	275.8	249.6	293.7	290.9	313.1	-
		038		187.6	223.9	256.2	236.1	267.4	268.3	285.9	-
		039		187.9	224.4	264.2	239.6	281.4	274.0	292.6	-
		040		177.5	216.5	255.9	240.4	271.1	268.0	293.1	-
		041		197.7	231.3	270.2	262.3	290.2	290.9	303.5	300.9
		042		187.4	228.6	259.5	243.0	269.5	273.4	290.9	289.7
		043		195.2	232.1	273.3	252.9	280.3	285.3	306.7	302.6
		044		183.6	229.0	266.9	243.7	287.0	285.6	299.3	304.1
		045		177.0	214.1	249.3	226.4	262.5	260.0	284.4	282.3

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Day:	Recovery 4	8	11	15	18	21
1	0 µg/day	001		-	-	-	-	-	-
		002		-	-	-	-	-	-
		003		-	-	-	-	-	-
		004		-	-	-	-	-	-
		005		-	-	-	-	-	-
		006		-	-	-	-	-	-
		007		-	-	-	-	-	-
		008		-	-	-	-	-	-
		009		-	-	-	-	-	-
		010		-	-	-	-	-	-
		011		320.0	328.2	331.7	328.6	333.4	341.3
		012		326.5	346.5	349.9	359.0	373.6	381.6
		013		291.7	297.5	303.2	308.8	314.5	317.1
		014		291.5	297.3	298.6	297.3	302.0	304.7
		015		350.7	363.2	370.3	374.3	383.6	394.7
2	30 µg/day	016		-	-	-	-	-	-
		017		-	-	-	-	-	-
		018		-	-	-	-	-	-
		019		-	-	-	-	-	-
		020		-	-	-	-	-	-
		021		-	-	-	-	-	-
		022		-	-	-	-	-	-
		023		-	-	-	-	-	-
		024		-	-	-	-	-	-
		025		-	-	-	-	-	-
		026		326.6	336.1	345.8	354.4	362.2	369.9
		027		312.9	327.4	335.6	344.3	347.4	359.0
		028		303.3	310.9	331.8	335.2	341.1	344.1
		029		323.2	337.7	350.5	355.5	361.9	371.9

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Day:	Recovery 4	8	11	15	18	21
2	30 µg/day	030		337.6	352.3	369.0	383.8	384.8	403.1
3	30 µg /day	031		-	-	-	-	-	-
		032		-	-	-	-	-	-
		033		-	-	-	-	-	-
		034		-	-	-	-	-	-
		035		-	-	-	-	-	-
		036		-	-	-	-	-	-
		037		-	-	-	-	-	-
		038		-	-	-	-	-	-
		039		-	-	-	-	-	-
		040		-	-	-	-	-	-
		041		312.3	327.1	331.7	340.8	351.0	357.8
		042		299.5	311.4	320.1	328.4	336.8	344.2
		043		313.6	331.7	336.5	344.4	355.5	362.5
		044		311.9	324.6	333.6	344.6	351.3	366.3
		045		293.5	308.8	316.1	315.8	326.1	340.4

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Day:	PID		Dosing					Recovery
				1	6	1	4	8	11	15	1
1	0 µg/day	046		151.3	169.9	188.5	177.4	192.7	202.2	206.7	-
		047		164.8	183.0	195.7	194.1	227.9	235.3	233.7	-
		048		156.5	170.4	185.8	172.6	195.1	187.6	190.0	-
		049		155.4	173.4	192.9	179.6	204.3	201.8	208.1	-
		050		149.6	174.8	185.3	182.4	218.0	227.9	227.0	-
		051		167.5	185.7	201.8	191.8	214.4	206.7	209.3	-
		052		158.8	176.0	206.3	190.6	213.7	213.3	220.6	-
		053		157.6	177.5	199.6	187.7	207.1	212.2	215.4	-
		054		166.0	186.8	203.7	193.6	211.0	225.7	230.7	-
		055		155.8	174.8	191.2	178.0	203.0	206.4	211.2	-
		056		166.8	181.7	203.6	188.4	211.9	210.5	219.0	224.3
		057		149.4	164.5	184.1	168.4	187.6	197.3	199.7	199.4
		058		151.7	169.0	187.0	172.2	186.8	197.4	204.0	204.8
		059		151.3	168.4	186.9	176.3	205.7	208.1	215.4	212.2
		060		173.1	189.5	209.5	194.7	218.7	221.1	223.6	234.7
2	30 µg/day	061		163.9	180.5	202.1	181.3	209.9	210.3	229.7	-
		062		157.5	172.7	183.1	174.6	203.0	205.8	208.9	-
		063		160.0	171.0	186.2	178.0	197.2	195.8	201.9	-
		064		162.7	184.5	198.0	187.2	215.7	208.9	216.1	-
		065		157.2	182.6	188.7	173.3	199.0	207.9	219.0	-
		066		171.4	186.7	203.9	187.0	215.0	215.8	219.6	-
		067		156.8	168.5	192.0	182.3	207.4	208.7	217.5	-
		068		152.0	169.9	187.6	169.0	190.5	187.7	196.9	-
		069		163.9	181.5	197.2	177.4	200.3	213.1	232.7	-
		070		174.1	188.9	202.5	185.8	206.0	211.6	227.2	-
		071		167.1	175.4	180.3	171.3	195.0	202.6	212.3	205.5
		072		153.5	174.3	196.8	177.1	207.9	198.7	213.4	206.6
		073		146.6	163.7	176.7	168.0	190.9	195.5	198.3	203.7
		074		154.4	168.3	186.3	173.0	195.0	192.4	196.3	204.8

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Day:	PID		Dosing					Recovery
				1	6	1	4	8	11	15	1
2	30 µg/day	075		156.4	176.2	191.5	174.4	204.8	203.4	220.5	215.5
3	30 µg /day	076		169.2	176.6	204.0	186.9	204.0	209.1	215.5	-
		077		155.0	167.5	181.6	171.1	184.4	194.2	213.2	-
		078		158.2	182.3	207.2	181.9	217.2	208.4	231.7	-
		079		159.0	175.8	194.7	175.2	191.4	206.9	227.1	-
		080		159.3	181.9	195.2	184.6	216.0	220.7	223.6	-
		081		158.6	168.4	192.4	178.0	194.7	201.5	208.6	-
		082		148.7	162.1	187.5	177.1	196.6	192.1	204.4	-
		083		154.1	166.2	187.3	175.1	197.5	204.6	207.0	-
		084		165.1	188.8	200.6	184.3	213.6	210.8	217.8	-
		085		159.2	174.0	189.3	168.8	187.3	193.9	199.1	-
		086		154.4	173.5	180.3	166.1	183.6	192.9	207.6	213.3
		087		162.2	180.8	198.4	182.2	204.0	215.8	241.3	242.5
		088		150.5	159.0	172.9	161.5	180.3	176.1	182.5	180.6
		089		170.6	185.8	204.4	184.1	211.3	209.9	218.4	208.1
		090		169.5	191.4	194.4	177.1	201.8	205.6	211.1	215.1

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Day:	Recovery 4	8	11	15	18	21
1	0 µg/day	046		-	-	-	-	-	-
		047		-	-	-	-	-	-
		048		-	-	-	-	-	-
		049		-	-	-	-	-	-
		050		-	-	-	-	-	-
		051		-	-	-	-	-	-
		052		-	-	-	-	-	-
		053		-	-	-	-	-	-
		054		-	-	-	-	-	-
		055		-	-	-	-	-	-
		056		234.4	243.0	241.5	238.7	238.8	246.9
		057		198.4	207.9	212.2	211.5	210.8	218.3
		058		207.6	210.0	204.5	213.9	211.2	219.1
		059		209.4	209.7	218.0	217.0	221.5	218.0
		060		235.9	249.5	243.9	240.1	245.4	242.0
2	30 µg/day	061		-	-	-	-	-	-
		062		-	-	-	-	-	-
		063		-	-	-	-	-	-
		064		-	-	-	-	-	-
		065		-	-	-	-	-	-
		066		-	-	-	-	-	-
		067		-	-	-	-	-	-
		068		-	-	-	-	-	-
		069		-	-	-	-	-	-
		070		-	-	-	-	-	-
		071		212.7	221.2	226.2	218.8	225.0	235.6
		072		217.4	227.3	224.6	225.6	224.6	242.1
		073		200.9	207.9	210.8	213.6	215.3	215.5
		074		219.4	221.2	217.4	217.4	222.8	226.7

Appendix 4

Body Weight (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Day:	Recovery 4	8	11	15	18	21
2	30 µg/day	075		214.6	223.1	228.5	227.5	235.1	236.7
3	30 µg /day	076		-	-	-	-	-	-
		077		-	-	-	-	-	-
		078		-	-	-	-	-	-
		079		-	-	-	-	-	-
		080		-	-	-	-	-	-
		081		-	-	-	-	-	-
		082		-	-	-	-	-	-
		083		-	-	-	-	-	-
		084		-	-	-	-	-	-
		085		-	-	-	-	-	-
		086		207.2	209.8	215.1	221.0	228.4	220.1
		087		235.5	239.1	233.2	241.0	252.5	246.1
		088		188.7	194.7	192.5	188.4	194.3	197.3
		089		217.4	225.8	226.0	222.2	229.6	234.3
		090		223.1	230.0	224.3	213.9	221.1	224.5

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Grp Num = Group Number; Animal Num = Animal Number; - = Value not applicable; NW = Not Weighed; e = Excluded.

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Days:	PID	Dosing					Recovery	
				1-6	1-4	4-8	8-11	11-15	1-15	1-4	4-8
1	0 µg/day	001		39.5	-13.8	31.4	13.8	5.8	37.2	-	-
		002		44.3	-12.2	36.0	14.3	17.7	55.8	-	-
		003		46.4	-11.1	31.9	15.8	11.8	48.4	-	-
		004		34.2	-6.8	45.5	8.4	21.7	68.8	-	-
		005		37.5	-9.8	32.4	15.0	14.4	52.0	-	-
		006		41.7	-10.2	37.4	11.8	16.2	55.2	-	-
		007		36.5	-10.9	35.7	14.6	11.6	51.0	-	-
		008		31.4	-13.5	31.7	9.5	13.2	40.9	-	-
		009		31.5	-7.6	26.8	8.6	17.1	44.9	-	-
		010		39.6	-14.2	33.4	10.4	17.0	46.6	-	-
		011		32.2	-7.0	34.1	5.6	13.4	46.1	6.2	8.2
		012		39.2	-34.1	-45.0	63.7	32.0	16.6	19.1	20.0
		013		30.3	-10.0	29.6	5.8	14.9	40.3	4.1	5.8
		014		36.0	-13.9	31.7	12.6	8.7	39.1	2.6	5.8
		015		43.2	-14.5	34.0	18.6	19.1	57.2	9.9	12.5
2	30 µg/day	016		32.4	-25.2	36.7	-3.6	18.7	26.6	-	-
		017		36.3	-17.4	41.7	0.9	14.9	40.1	-	-
		018		40.3	-13.2	29.4	0.4	14.5	31.1	-	-
		019		39.6	-10.7	28.8	5.2	17.2	40.5	-	-
		020		38.6	-22.9	35.5	-4.1	14.7	23.2	-	-
		021		43.2	-20.5	30.3	-2.7	19.5	26.6	-	-
		022		41.7	-21.9	32.7	-0.4	22.1	32.5	-	-
		023		38.0	-18.5	31.6	8.2	14.9	36.2	-	-
		024		38.3	-23.0	33.3	-6.7	14.4	18.0	-	-
		025		39.8	-16.6	43.6	-3.7	17.6	40.9	-	-
		026		38.8	-25.2	44.1	4.8	19.1	42.8	15.7	9.5
		027		35.4	-22.4	43.0	-2.4	22.3	40.5	6.9	14.5
		028		30.2	-18.4	32.5	-1.6	24.4	36.9	13.3	7.6

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Days:	PID	Dosing					Recovery	
				1-6	1-4	4-8	8-11	11-15	1-15	1-4	4-8
2	30 µg/day	029		43.7	-20.4	35.5	2.3	23.0	40.4	10.4	14.5
		030		36.3	-17.3	41.4	4.9	25.0	54.0	14.8	14.7
3	30 µg /day	031		25.0	-19.7	25.9	-0.1	18.4	24.5	-	-
		032		32.6	-19.9	29.4	-5.8	16.1	19.8	-	-
		033		42.1	-21.8	41.0	5.0	14.7	38.9	-	-
		034		34.9	-25.8	33.9	-3.2	15.9	20.8	-	-
		035		39.1	-29.3	32.2	-13.8	22.2	11.3	-	-
		036		44.8	-20.0	34.8	-1.1	20.2	33.9	-	-
		037		42.7	-26.2	44.1	-2.8	22.2	37.3	-	-
		038		36.3	-20.1	31.3	0.9	17.6	29.7	-	-
		039		36.5	-24.6	41.8	-7.4	18.6	28.4	-	-
		040		39.0	-15.5	30.7	-3.1	25.1	37.2	-	-
		041		33.6	-7.9	27.9	0.7	12.6	33.3	11.4	14.8
		042		41.2	-16.5	26.5	3.9	17.5	31.4	9.8	11.9
		043		36.9	-20.4	27.4	5.0	21.4	33.4	11.0	18.1
		044		45.4	-23.2	43.3	-1.4	13.7	32.4	7.8	12.7
		045		37.1	-22.9	36.1	-2.5	24.4	35.1	11.2	15.3

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Days:	Recovery 8-11	11-15	15-18	18-21	1-21
1	0 µg/day	001		-	-	-	-	-
		002		-	-	-	-	-
		003		-	-	-	-	-
		004		-	-	-	-	-
		005		-	-	-	-	-
		006		-	-	-	-	-
		007		-	-	-	-	-
		008		-	-	-	-	-
		009		-	-	-	-	-
		010		-	-	-	-	-
		011		3.5	-3.1	4.8	7.9	27.5
		012		3.4	9.1	14.6	8.0	74.2
		013		5.7	5.6	5.7	2.6	29.5
		014		1.3	-1.3	4.7	2.7	15.8
		015		7.1	4.0	9.3	11.1	53.9
2	30 µg/day	016		-	-	-	-	-
		017		-	-	-	-	-
		018		-	-	-	-	-
		019		-	-	-	-	-
		020		-	-	-	-	-
		021		-	-	-	-	-
		022		-	-	-	-	-
		023		-	-	-	-	-
		024		-	-	-	-	-
		025		-	-	-	-	-
		026		9.7	8.6	7.8	7.7	59.0
		027		8.2	8.7	3.1	11.6	53.0
		028		20.9	3.4	5.9	3.0	54.1

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase Days:	Recovery 8-11	11-15	15-18	18-21	1-21
2	30 µg/day	029		12.8	5.0	6.4	10.0	59.1
		030		16.7	14.8	1.0	18.3	80.3
3	30 µg /day	031		-	-	-	-	-
		032		-	-	-	-	-
		033		-	-	-	-	-
		034		-	-	-	-	-
		035		-	-	-	-	-
		036		-	-	-	-	-
		037		-	-	-	-	-
		038		-	-	-	-	-
		039		-	-	-	-	-
		040		-	-	-	-	-
		041		4.6	9.1	10.2	6.8	56.9
		042		8.7	8.3	8.4	7.4	54.5
		043		4.8	7.9	11.1	7.0	59.9
		044		9.0	11.0	6.7	15.0	62.2
		045		7.3	-0.3	10.3	14.3	58.1

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Days:	PID	Dosing					Recovery	
				1-6	1-4	4-8	8-11	11-15	1-15	1-4	4-8
1	0 µg/day	046		18.6	-11.1	15.3	9.5	4.5	18.2	-	-
		047		18.2	-1.6	33.8	7.4	-1.6	38.0	-	-
		048		13.9	-13.2	22.5	-7.5	2.4	4.2	-	-
		049		18.0	-13.3	24.7	-2.5	6.3	15.2	-	-
		050		25.2	-2.9	35.6	9.9	-0.9	41.7	-	-
		051		18.2	-10.0	22.6	-7.7	2.6	7.5	-	-
		052		17.2	-15.7	23.1	-0.4	7.3	14.3	-	-
		053		19.9	-11.9	19.4	5.1	3.2	15.8	-	-
		054		20.8	-10.1	17.4	14.7	5.0	27.0	-	-
		055		19.0	-13.2	25.0	3.4	4.8	20.0	-	-
		056		14.9	-15.2	23.5	-1.4	8.5	15.4	10.1	8.6
		057		15.1	-15.7	19.2	9.7	2.4	15.6	-1.0	9.5
		058		17.3	-14.8	14.6	10.6	6.6	17.0	2.8	2.4
		059		17.1	-10.6	29.4	2.4	7.3	28.5	-2.8	0.3
		060		16.4	-14.8	24.0	2.4	2.5	14.1	1.2	13.6
2	30 µg/day	061		16.6	-20.8	28.6	0.4	19.4	27.6	-	-
		062		15.2	-8.5	28.4	2.8	3.1	25.8	-	-
		063		11.0	-8.2	19.2	-1.4	6.1	15.7	-	-
		064		21.8	-10.8	28.5	-6.8	7.2	18.1	-	-
		065		25.4	-15.4	25.7	8.9	11.1	30.3	-	-
		066		15.3	-16.9	28.0	0.8	3.8	15.7	-	-
		067		11.7	-9.7	25.1	1.3	8.8	25.5	-	-
		068		17.9	-18.6	21.5	-2.8	9.2	9.3	-	-
		069		17.6	-19.8	22.9	12.8	19.6	35.5	-	-
		070		14.8	-16.7	20.2	5.6	15.6	24.7	-	-
		071		8.3	-9.0	23.7	7.6	9.7	32.0	7.2	8.5
		072		20.8	-19.7	30.8	-9.2	14.7	16.6	10.8	9.9
		073		17.1	-8.7	22.9	4.6	2.8	21.6	-2.8	7.0

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Days:	PID	Dosing					Recovery	
				1-6	1-4	4-8	8-11	11-15	1-15	1-4	4-8
2	30 µg/day	074		13.9	-13.3	22.0	-2.6	3.9	10.0	14.6	1.8
		075		19.8	-17.1	30.4	-1.4	17.1	29.0	-0.9	8.5
3	30 µg /day	076		7.4	-17.1	17.1	5.1	6.4	11.5	-	-
		077		12.5	-10.5	13.3	9.8	19.0	31.6	-	-
		078		24.1	-25.3	35.3	-8.8	23.3	24.5	-	-
		079		16.8	-19.5	16.2	15.5	20.2	32.4	-	-
		080		22.6	-10.6	31.4	4.7	2.9	28.4	-	-
		081		9.8	-14.4	16.7	6.8	7.1	16.2	-	-
		082		13.4	-10.4	19.5	-4.5	12.3	16.9	-	-
		083		12.1	-12.2	22.4	7.1	2.4	19.7	-	-
		084		23.7	-16.3	29.3	-2.8	7.0	17.2	-	-
		085		14.8	-20.5	18.5	6.6	5.2	9.8	-	-
		086		19.1	-14.2	17.5	9.3	14.7	27.3	-6.1	2.6
		087		18.6	-16.2	21.8	11.8	25.5	42.9	-7.0	3.6
		088		8.5	-11.4	18.8	-4.2	6.4	9.6	8.1	6.0
		089		15.2	-20.3	27.2	-1.4	8.5	14.0	9.3	8.4
		090		21.9	-17.3	24.7	3.8	5.5	16.7	8.0	6.9

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase Days:	Recovery 8-11	11-15	15-18	18-21	1-21
1	0 µg/day	046		-	-	-	-	-
		047		-	-	-	-	-
		048		-	-	-	-	-
		049		-	-	-	-	-
		050		-	-	-	-	-
		051		-	-	-	-	-
		052		-	-	-	-	-
		053		-	-	-	-	-
		054		-	-	-	-	-
		055		-	-	-	-	-
		056		-1.5	-2.8	0.1	8.1	22.6
		057		4.3	-0.7	-0.7	7.5	18.9
		058		-5.5	9.4	-2.7	7.9	14.3
		059		8.3	-1.0	4.5	-3.5	5.8
		060		-5.6	-3.8	5.3	-3.4	7.3
2	30 µg/day	061		-	-	-	-	-
		062		-	-	-	-	-
		063		-	-	-	-	-
		064		-	-	-	-	-
		065		-	-	-	-	-
		066		-	-	-	-	-
		067		-	-	-	-	-
		068		-	-	-	-	-
		069		-	-	-	-	-
		070		-	-	-	-	-
		071		5.0	-7.4	6.2	10.6	30.1
		072		-2.7	1.0	-1.0	17.5	35.5
		073		2.9	2.8	1.7	0.2	11.8

Appendix 5

Body Weight Change During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase	Recovery				
			Days:	8-11	11-15	15-18	18-21	1-21
2	30 µg/day	074		-3.8	0.0	5.4	3.9	21.9
		075		5.4	-1.0	7.6	1.6	21.2
3	30 µg /day	076		-	-	-	-	-
		077		-	-	-	-	-
		078		-	-	-	-	-
		079		-	-	-	-	-
		080		-	-	-	-	-
		081		-	-	-	-	-
		082		-	-	-	-	-
		083		-	-	-	-	-
		084		-	-	-	-	-
		085		-	-	-	-	-
		086		5.3	5.9	7.4	-8.3	6.8
		087		-5.9	7.8	11.5	-6.4	3.6
		088		-2.2	-4.1	5.9	3.0	16.7
		089		0.2	-3.8	7.4	4.7	26.2
		090		-5.7	-10.4	7.2	3.4	9.4

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

Num = Number; - = Value not applicable; NW = Not Weighed; e = Excluded; SP = Spilled.

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
1	0 µg/day	001		53.4	92.3	66.2	87.0	298.9	-	-	-
		002		53.5	100.9	66.5	92.1	313.0	-	-	-
		003		53.6	92.7	70.2	85.0	301.5	-	-	-
		004		51.8	106.3	66.5	96.8	321.4	-	-	-
		005		50.4	95.2	67.9	87.4	300.9	-	-	-
		006		54.3	100.3	62.6	95.1	312.3	-	-	-
		007		54.4	106.8	70.4	92.3	323.9	-	-	-
		008		45.3	89.9	64.8	84.1	284.1	-	-	-
		009		50.5	86.2	58.9	87.2	282.8	-	-	-
		010		46.8	88.5	67.6	87.4	290.3	-	-	-
		011		51.5	97.4	59.9	85.1	293.9	48.2	74.0	55.8
		012		39.7	18.5	58.5	101.2	217.9	56.2	97.1	63.2
		013		51.9	85.1	53.2	81.5	271.7	39.6	72.9	52.6
		014		53.3	95.8	69.8	84.2	303.1	44.4	75.5	52.0
		015		52.8	107.1	68.5	93.9	322.3	51.7	91.1	67.0
2	30 µg/day	016		35.5	85.3	47.2	81.5	249.5	-	-	-
		017		43.1	95.9	53.2	97.7	289.9	-	-	-
		018		46.1	101.9	57.4	88.9	294.3	-	-	-
		019		45.0	92.8	49.2	86.1	273.1	-	-	-
		020		40.7	91.7	47.0	84.9	264.3	-	-	-
		021		34.4	89.8	46.4	91.1	261.7	-	-	-

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
2	30 µg/day	022		41.2	90.7	49.3	84.6	265.8	-	-	-
		023		42.2	89.0	54.1	84.2	269.5	-	-	-
		024		38.6	90.5	51.2	86.2	266.5	-	-	-
		025		51.1	114.3	61.9	104.3	331.6	-	-	-
		026		43.6	101.5	65.9	101.9	312.9	63.9	94.9	65.3
		027		46.4	101.0	57.0	90.9	295.3	62.8	89.6	63.3
		028		41.9	90.8	50.7	92.3	275.7	61.3	84.0	66.5
		029		45.7	107.5	57.2	97.8	308.2	65.6	90.9	68.2
		030		43.4	108.6	62.6	110.9	325.5	70.1	105.2	76.7
3	30 µg /day	031		33.4	75.7	45.2	74.5	228.8	-	-	-
		032		40.9	85.0	41.1	86.8	253.8	-	-	-
		033		36.3	116.5	63.6	100.2	316.6	-	-	-
		034		36.3	84.9	47.1	80.6	248.9	-	-	-
		035		33.4	80.0	35.7	80.5	229.6	-	-	-
		036		42.4	87.4	55.1	89.2	274.1	-	-	-
		037		39.4	105.8	56.7	103.3	305.2	-	-	-
		038		39.2	79.7	52.5	82.9	254.3	-	-	-
		039		33.8	97.4	49.6	87.1	267.9	-	-	-
		040		38.6	95.1	44.6	101.0	279.3	-	-	-
		041		53.9	105.4	61.5	93.1	313.9	70.0	94.5	67.3
		042		34.0	91.5	46.9	82.2	254.6	59.8	79.8	56.9

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
3	30 µg /day	043		39.6	78.2	50.6	86.5	254.9	58.4	83.2	61.6
		044		38.7	98.2	55.6	94.1	286.6	63.2	92.5	61.6
		045		40.4	89.8	50.9	90.0	271.1	59.9	83.2	66.1

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	Recovery 15-18	Recovery 18-21	Recovery 1-21
1	0 µg/day	001		-	-	-	-
		002		-	-	-	-
		003		-	-	-	-
		004		-	-	-	-
		005		-	-	-	-
		006		-	-	-	-
		007		-	-	-	-
		008		-	-	-	-
		009		-	-	-	-
		010		-	-	-	-
		011		69.1	53.3	50.5	350.9
		012		87.6	68.2	66.9	439.2
		013		68.6	54.3	51.6	339.6
		014		70.9	53.6	56.9	353.3
		015		85.9	69.1	70.5	435.3
2	30 µg/day	016		-	-	-	-
		017		-	-	-	-
		018		-	-	-	-
		019		-	-	-	-
		020		-	-	-	-
		021		-	-	-	-

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	15-18	18-21	1-21
2	30 µg/day	022		-	-	-	-
		023		-	-	-	-
		024		-	-	-	-
		025		-	-	-	-
		026		87.1	68.5	67.9	447.6
		027		82.1	64.7	64.1	426.6
		028		75.1	59.3	63.9	410.1
		029		82.8	62.2	63.3	433.0
		030		96.5	67.6	73.0	489.1
3	30 µg /day	031		-	-	-	-
		032		-	-	-	-
		033		-	-	-	-
		034		-	-	-	-
		035		-	-	-	-
		036		-	-	-	-
		037		-	-	-	-
		038		-	-	-	-
		039		-	-	-	-
		040		-	-	-	-
		041		85.4	70.3	70.0	457.5
		042		71.6	58.4	53.6	380.1

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	15-18	18-21	1-21
3	30 µg /day	043		76.1	58.4	56.5	394.2
		044		85.8	65.0	69.8	437.9
		045		77.1	60.9	60.8	408.0

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
1	0 µg/day	046		32.9	61.5	47.7	54.5	196.6	-	-	-
		047		41.0	87.3	61.3	77.0	266.6	-	-	-
		048		35.6	72.7	44.7	64.2	217.2	-	-	-
		049		36.3	72.0	43.4	63.5	215.2	-	-	-
		050		42.8	83.1	59.2	69.6	254.7	-	-	-
		051		37.5	67.9	42.3	67.2	214.9	-	-	-
		052		43.8	79.3	46.3	64.0	233.4	-	-	-
		053		39.1	74.0	47.1	61.9	222.1	-	-	-
		054		41.8	84.0	58.1	77.5	261.4	-	-	-
		055		35.1	68.4	46.2	59.1	208.8	-	-	-
		056		35.8	75.7	47.3	72.5	231.3	54.1	70.3	51.0
		057		28.2	64.0	39.5	55.0	186.7	43.2	62.4	45.0
		058		34.8	63.2	42.7	56.1	196.8	42.4	55.2	40.0
		059		40.2	81.0	44.7	65.8	231.7	45.2	56.7	45.7
		060		42.0	82.8	53.6	71.2	249.6	53.1	72.0	49.9
2	30 µg/day	061		30.3	74.3	44.2	80.6	229.4	-	-	-
		062		34.0	65.9	40.3	61.5	201.7	-	-	-
		063		36.7	70.6	38.7	66.5	212.5	-	-	-
		064		36.6	77.5	46.5	71.6	232.2	-	-	-
		065		23.9	72.6	42.7	66.2	205.4	-	-	-
		066		29.5	71.5	41.1	56.8	198.9	-	-	-

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
2	30 µg/day	067		37.3	78.2	45.8	68.4	229.7	-	-	-
		068		30.0	59.8	37.7	56.7	184.2	-	-	-
		069		34.0	71.0	44.2	72.5	221.7	-	-	-
		070		32.9	70.8	41.7	71.7	217.1	-	-	-
		071		37.4	72.6	40.4	66.9	217.3	55.6	67.9	51.2
		072		34.0	71.0	38.4	66.1	209.5	47.7	68.7	47.1
		073		32.9	72.4	39.6	63.5	208.4	44.2	63.3	44.1
		074		31.4	67.3	40.0	58.1	196.8	54.2	63.8	41.9
		075		34.4	66.9	46.5	71.7	219.5	46.9	69.7	49.2
3	30 µg /day	076		38.5	76.7	50.1	70.3	235.6	-	-	-
		077		34.2	71.1	40.5	77.0	222.8	-	-	-
		078		25.5	74.6	36.2	72.1	208.4	-	-	-
		079		30.1	67.5	43.8	76.3	217.7	-	-	-
		080		37.3	82.4	55.1	74.3	249.1	-	-	-
		081		31.8	64.5	34.7	65.3	196.3	-	-	-
		082		31.7	66.9	37.4	62.8	198.8	-	-	-
		083		33.3	71.5	45.0	66.8	216.6	-	-	-
		084		78.1	75.9	39.0	62.1	255.1	-	-	-
		085		29.8	66.7	39.3	66.7	202.5	-	-	-
		086		27.3	64.9	36.4	62.6	191.2	47.8	60.3	42.8
		087		38.6	85.4	43.6	90.6	258.2	57.6	82.6	53.6

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Dosing					Recovery		
				1-4	4-8	8-11	11-15	1-15	1-4	4-8	8-11
3	30 µg /day	088		26.7	65.5	31.8	54.8	178.8	45.4	62.4	38.3
		089		28.5	64.7	37.4	60.1	190.7	44.1	59.9	42.1
		090		28.1	77.6	36.0	65.7	207.4	50.2	69.2	36.8

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	15-18	18-21	1-21
1	0 µg/day	046		-	-	-	-
		047		-	-	-	-
		048		-	-	-	-
		049		-	-	-	-
		050		-	-	-	-
		051		-	-	-	-
		052		-	-	-	-
		053		-	-	-	-
		054		-	-	-	-
		055		-	-	-	-
		056		67.1	47.3	50.0	339.8
		057		56.2	39.3	46.0	292.1
		058		52.0	38.9	45.2	273.7
		059		52.6	36.9	38.6	275.7
		060		67.5	49.8	45.2	337.5
2	30 µg/day	061		-	-	-	-
		062		-	-	-	-
		063		-	-	-	-
		064		-	-	-	-
		065		-	-	-	-
		066		-	-	-	-

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	15-18	18-21	1-21
2	30 µg/day	067		-	-	-	-
		068		-	-	-	-
		069		-	-	-	-
		070		-	-	-	-
		071		58.4	46.1	48.6	327.8
		072		63.9	36.2	48.1	311.7
		073		55.4	40.2	41.1	288.3
		074		61.4	44.0	44.4	309.7
		075		65.8	51.7	51.8	335.1
3	30 µg /day	076		-	-	-	-
		077		-	-	-	-
		078		-	-	-	-
		079		-	-	-	-
		080		-	-	-	-
		081		-	-	-	-
		082		-	-	-	-
		083		-	-	-	-
		084		-	-	-	-
		085		-	-	-	-
		086		60.1	44.2	41.1	296.3
		087		77.3	57.0	55.9	384.0

Appendix 6

Food Consumption - Empty Feeder During Interval (g)

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Grp Num	Dose	Animal Num	Phase: Days:	Recovery 11-15	15-18	18-21	1-21
3	30 µg /day	088		50.3	37.6	40.0	274.0
		089		52.8	41.5	42.6	283.0
		090		48.9	43.5	44.2	292.8

Appendix 7

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description	Parameter	Description
RBC	Red Blood Cells	BASO	Basophil, Absolute
HGB	Hemoglobin	BASO_P	Basophil
HCT	Hematocrit	LUC	Large Unstained Cells, Absolute
MCV	Mean Cell Volume	LUC_P	Large Unstained Cells
MCH	Mean Cell Hemoglobin	MORPH	Morphology
MCHC	Mean Cell Hemoglobin Conc	POIK	Poikilocytosis
RDW	Red Cell Distribution Width	BURR	Burr Cells
RETIC_P	Reticulocyte	SCHISTO	Schistocytes
RETIC	Reticulocyte, Absolute	SPHERO	Spherocytes
PLT	Platelets	SIDERO	Siderocyte-like Inclusions
MPV	Mean Platelet Volume	TARGET	Target Cells
WBC	White Blood Cells	TEAR	Tear Drop Cells
NEUT	Neutrophil, Absolute	B_STIP	Basophilic Stippling
NEUT_P	Neutrophil	HJ	Howell-Jolly Bodies
LYM	Lymphocyte, Absolute	AGGL	Agglutination
LYM_P	Lymphocyte	CLPLT	Clumped Platelets
MONO	Monocyte, Absolute	HGB_CRYS	Hemoglobin Crystals
MONO_P	Monocyte	BASOPH	Basophilia
EO	Eosinophil, Absolute	ACANTH	Acanthocytes
EO_P	Eosinophil	STOM	Stomatocytes

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Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
OTHERM	Other Morphology
DOHLE	Dohle Bodies
HYSEG	Hypersegmented Neutrophils
TOXIC_G	Toxic Granulation
TOXIC_V	Toxic Vacuolation
VACLYM	Vacuolated Lymphocytes
PT_Rat	Prothrombin Time, Rat
APTT	Activated Partial Thromboplastin Time
FIB	Fibrinogen

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Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Comment	Description
CE	Critical result for Subject # RatWis042 ; Time point R022 transmitted electronically to clinically responsible personnel.
CE	Critical result for Subject # RatWis044 ; Time point R022 transmitted electronically to clinically responsible personnel.
CL	Clotted
FT	Subject# Ratwis031; Timepoint D004; Lymphocytes
NS	No Sample
QN	Quantity Not Sufficient
RP	Clumped platelets on original result; sample redrawn and reported.
RR	Result repeated
RW	RDW and MCV not reportable due to abnormal cytogram
SR	Slide Reviewed

Appendix 7

Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

- Value not applicable;

Note: Each interval/day will be concatenated with the phase name abbreviation

HPD = Hours Post Dose; U = Unscheduled

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %
1	0 µg/day	001	4D	--	8.64	15.9	50.0	57.8	18.4	31.7	11.6
			17D	-	8.38	14.9	46.2	55.2	17.8	32.2	11.1
		002	4D	--	8.07	15.7	48.7	60.4	19.4	32.2	12.1
			17D	-	7.33	13.8	42.8	58.3	18.8	32.2	11.7
		003	4D	--	8.11	15.0	49.2	60.6	18.5	30.5	12.1
			17D	-	- CL	- CL	- CL	- CL	- CL	- CL	- CL
		004	4D	--	8.17	14.8	48.0	58.8	18.2	30.9	13.0
			17D	-	7.63	14.0	42.6	55.8	18.4	32.9	11.8
		005	4D	--	8.02	14.4	46.4	57.8	18.0	31.1	12.7
			17D	-	7.06	12.7	38.8	55.0	18.0	32.8	12.3
		006	4D	--	8.05	14.6	47.3	58.7	18.2	30.9	12.4
			17D	-	7.95	14.2	44.4	55.8	17.9	32.0	12.0
		007	4D	--	7.76	14.7	46.7	60.2	18.9	31.4	12.0
			17D	-	7.43	13.7	42.3	57.0	18.4	32.3	11.7
		008	17D	-	8.10	14.5	45.1	55.7	18.0	32.2	11.6
		009	17D	-	6.75	12.8	39.4	58.5	19.0	32.5	11.4
		010	17D	-	7.63	13.8	41.9	54.9	18.1	33.0	11.1

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL		MCH pg	MCHC g/dL	RDW %	
1	0 µg/day	011	22R	-	7.29	12.8	37.9	52.1		17.6	33.8	12.2	
		012	22R	-	8.02	14.2	43.8	54.6		17.7	32.5	12.1	
		013	22R	-	8.61	15.5	46.4	-	RW	18.0	33.4	-	RW
		014	22R	-	8.06	15.0	43.6	54.2		18.6	34.4	11.3	
		015	22R	-	7.77	14.3	42.2	54.3		18.4	33.9	12.1	
2	30 µg/day	016	4D	--	8.30	15.0	46.2	55.7		18.1	32.5	11.9	
			17D	-	7.03	12.2	36.9	52.5		17.3	33.0	13.3	
		017	4D	--	7.60	13.4	41.4	54.5		17.6	32.3	13.1	
			17D	-	6.76	11.3	35.1	51.9		16.8	32.4	14.0	
		018	4D	--	7.71	13.9	42.0	54.5		18.0	33.1	13.8	
			17D	-	7.52	13.0	38.9	51.8		17.3	33.4	15.5	
		019	4D	--	7.56	14.1	43.4	57.4		18.7	32.6	12.4	
			17D	-	7.20	12.9	39.5	54.9		17.9	32.6	13.5	
		020	4D	--	7.92	14.0	43.3	54.7		17.7	32.4	12.9	
			17D	-	7.57	12.9	39.7	52.4		17.0	32.5	13.4	
		021	4D	--	7.43	13.7	42.4	57.0		18.4	32.3	13.5	
			17D	-	6.66	11.7	36.5	54.8		17.6	32.2	14.8	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL		MCH pg	MCHC g/dL	RDW %	
2	30 µg/day	022	4D	--	7.90	15.0	44.9	56.9		18.9	33.3	12.2	
			17D	-	7.21	13.1	39.5	54.7		18.2	33.3	14.1	
		023	17D	-	7.35	12.4	39.0	53.1		16.9	31.7	14.3	
		024	17D	-	7.16	12.6	39.0	54.5		17.6	32.4	14.8	
		025	17D	-	7.23	13.2	39.9	55.2		18.2	33.0	13.5	
		026	22R	-	8.26	14.0	43.6	52.8		17.0	32.2	13.5	
		027	22R	-	8.35	15.0	46.0	55.2		17.9	32.5	13.5	
		028	22R	-	8.11	14.1	43.5	53.6		17.4	32.4	13.1	
		029	22R	-	7.87	14.6	43.5	-	RW	18.5	33.6	-	RW
		030	22R	-	7.73	14.2	42.0	54.4		18.4	33.8	13.8	
3	30 µg /day	031	4D	--	7.62	13.5	42.3	55.6		17.8	32.0	11.8	
			17D	-	6.93	11.9	36.9	53.2		17.2	32.3	12.9	
		032	4D	--	7.23	13.8	42.9	59.4		19.1	32.2	13.3	
			17D	-	7.02	12.6	39.6	56.5		17.9	31.8	14.1	
		033	4D	--	7.41	13.8	43.6	58.9		18.7	31.7	12.7	
			17D	-	7.40	13.3	41.9	56.6		17.9	31.7	13.9	
		034	4D	--	7.79	14.1	43.6	56.0		18.1	32.3	12.1	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL		MCH pg	MCHC g/dL	RDW %	
3	30 µg /day	034	17D	-	7.42	13.0	39.7	53.5		17.5	32.8	13.6	
		035	4D	--	7.85	14.4	45.4	57.7		18.3	31.7	12.2	
			17D	-	7.34	13.2	40.1	54.7		18.0	33.0	13.9	
		036	4D	--	7.93	14.6	45.3	57.1		18.5	32.3	12.4	
			17D	-	7.46	13.3	40.6	54.4		17.8	32.6	13.2	
		037	4D	--	7.34	13.9	43.4	59.1		19.0	32.1	12.6	
			17D	-	6.88	12.5	38.8	56.5		18.1	32.1	13.6	
		038	17D	-	6.77	12.3	37.7	55.6		18.2	32.8	14.0	
		039	17D	-	7.37	13.3	39.7	53.9		18.0	33.4	14.4	
		040	17D	-	6.54	12.7	37.9	-	RW	19.5	33.6	-	RW
		041	22R	-	8.36	14.7	45.9	55.0		17.6	32.1	14.0	
		042	22R	-	7.62	13.8	42.5	55.7		18.1	32.5	13.1	
		043	22R	-	7.91	13.6	41.9	53.1		17.2	32.4	13.2	
		044	22R	-	8.22	14.2	43.9	53.4		17.3	32.3	13.0	
		045	22R	-	7.32	13.7	40.7	-	RW	18.8	33.7	-	RW

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL	MPV fL	WBC 10e3/uL	NEUT 10^3/uL	NEUT_P %
1	0 µg/day	001	4D	--	4.6	397	1132	8.3	7.5	0.92	12.2
			17D	-	2.4	201	870	8.3	3.7	0.75	20.3
		002	4D	--	4.8	387	963	8.9	5.6	0.97	17.3
			17D	-	2.5	183	873	9.1	3.9	0.70	17.9
		003	4D	--	5.1	414	1154	9.1	7.4	0.58	7.8
			17D	-	- CL	- CL	- CL	- CL	- CL	- CL	- CL
		004	4D	--	5.5	449	1099	8.9	8.4	1.43	16.9
			17D	-	2.5	191	958	9.3	5.4	1.29	24.1
		005	4D	--	5.3	425	1069	8.5	8.8	0.68	7.7
			17D	-	2.7	191	927	9.1	1.9	0.18	9.4
		006	4D	--	4.8	386	1014	9.2	7.1	1.24	17.3
			17D	-	2.0	159	905	9.5	5.4	1.08	19.8
		007	4D	--	3.7	287	658	9.2	8.4	1.76	21.0
			17D	-	1.7	126	918	8.9	6.3	0.99	15.7
		008	17D	-	2.4	194	794	9.5	4.1	0.43	10.3
		009	17D	-	2.9	196	750	9.2	1.5	0.29	19.6
		010	17D	-	2.2	168	937	9.2	2.4	0.36	15.2

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL	MPV fL	WBC 10e3/uL	NEUT 10^3/uL	NEUT_P %
1	0 µg/day	011	22R	-	2.5	182	909	9.2	2.2	0.60	27.6
		012	22R	-	2.8	225	834	9.2	4.9	0.93	19.1
		013	22R	-	2.1	181	865	8.8	9.5	1.49	15.7
		014	22R	-	1.8	145	809	8.7	4.6	0.56	12.1
		015	22R	-	2.2	171	822	9.1	5.1	0.91	17.9
2	30 µg/day	016	4D	--	1.3	108	910	9.9	13.0	2.97	22.8
			17D	-	2.9	204	564	10.6	6.4	3.27	50.8
		017	4D	--	0.7	53	1143	9.2	12.6	2.90	23.1
			17D	-	2.7	183	927	9.4	8.8	4.96	56.1
		018	4D	--	0.8	62	1044	9.5	14.3	2.83	19.8
			17D	-	2.0	150	815	9.4	14.5	6.56	45.1
		019	4D	--	2.4	181	1004	9.2	7.3	1.67	22.9
			17D	-	2.5	180	855	9.3	7.4	3.76	51.1
		020	4D	--	1.0	79	1177	8.9	12.0	3.24	27.0
			17D	-	2.2	167	880	9.0	12.8	7.32	57.0
		021	4D	--	1.6	119	1039	9.6	6.6	0.95	14.3
			17D	-	2.3	153	655	9.7	3.5	1.45	41.3

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL		MPV fL		WBC 10e3/uL	NEUT 10^3/uL	NEUT_P %
2	30 µg/day	022	4D	--	1.9	150	961	RP	7.7	RP	9.1	2.73	30.1
			17D	-	2.5	180	926		9.8		9.4	4.80	50.8
		023	17D	-	2.6	191	872		9.3		4.0	1.86	46.9
		024	17D	-	3.0	215	724		9.9		9.6	4.94	51.5
		025	17D	-	3.2	231	795		9.1		11.9	5.57	46.7
		026	22R	-	2.7	223	816		8.7		4.3	1.19	27.7
		027	22R	-	2.2	184	910		8.9		6.4	1.14	17.8
		028	22R	-	2.7	219	769		9.1		5.5	0.69	12.5
		029	22R	-	1.9	150	1054		9.0		6.3	1.20	19.0
		030	22R	-	2.3	178	973		8.5		7.4	1.13	15.3
3	30 µg /day	031	4D	--	1.5	114	898		9.6		7.6	1.38	18.0
			17D	-	2.8	194	534	SR	10.1		6.7	3.36	49.9
		032	4D	--	1.5	108	937		10.3		11.1	2.60	23.5
			17D	-	2.9	204	807		10.1		10.5	5.08	48.3
		033	4D	--	1.0	74	1187		9.6		12.3	2.89	23.5
			17D	-	2.4	178	974		9.3		9.2	5.03	54.6
		034	4D	--	1.3	101	843		10.1		9.2	2.07	22.4

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL		MPV fL	WBC 10e3/uL	NEUT 10^3/uL	NEUT_P %
3	30 µg /day	034	17D	-	2.6	193	708		10.3	8.0	4.24	52.9
		035	4D	--	1.6	126	780		9.6	8.1	1.66	20.6
			17D	-	2.8	206	594	SR	10.5	7.8	3.76	48.2
		036	4D	--	1.8	143	1024		9.3	10.0	2.34	23.3
			17D	-	2.7	201	908		9.8	10.1	5.34	52.8
		037	4D	--	0.9	66	947		9.4	9.6	2.19	22.7
			17D	-	2.5	172	667		9.6	8.0	3.82	47.6
		038	17D	-	3.0	203	702		10.8	9.1	4.57	50.2
		039	17D	-	2.8	206	759		9.4	8.6	4.72	54.9
		040	17D	-	2.8	183	737		9.4	8.0	3.59	44.7
		041	22R	-	2.4	201	789		9.2	4.9	1.36	27.7
		042	22R	-	2.6	198	689		9.1	2.9	0.70	23.9
		043	22R	-	2.1	166	822		8.7	5.9	1.37	23.3
		044	22R	-	2.6	214	890		8.8	5.2	1.53	29.6
		045	22R	-	2.1	154	998		8.6	5.6	1.42	25.4

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
1	0 µg/day	001	4D	--	6.35	84.4	0.12	1.5	0.07	0.9	0.02
			17D	-	2.80	75.4	0.03	0.9	0.09	2.6	0.00
		002	4D	--	4.46	79.1	0.12	2.2	0.03	0.6	0.01
			17D	-	3.00	76.9	0.13	3.2	0.04	1.0	0.00
		003	4D	--	6.60	89.3	0.11	1.5	0.06	0.8	0.01
			17D	-	- CL	- CL	- CL	- CL	- CL	- CL	- CL
		004	4D	--	6.80	80.6	0.09	1.0	0.06	0.7	0.01
			17D	-	3.85	71.8	0.12	2.3	0.06	1.1	0.01
		005	4D	--	7.86	89.2	0.12	1.4	0.08	0.9	0.02
			17D	-	1.66	87.1	0.03	1.5	0.03	1.3	0.00
		006	4D	--	5.64	79.2	0.13	1.8	0.06	0.8	0.02
			17D	-	4.12	75.6	0.11	2.1	0.09	1.7	0.01
		007	4D	--	6.28	74.9	0.07	0.8	0.21	2.5	0.02
			17D	-	5.09	81.2	0.08	1.2	0.06	1.0	0.01
		008	17D	-	3.53	85.4	0.08	2.0	0.06	1.5	0.00
		009	17D	-	1.14	77.0	0.03	2.2	0.02	1.1	0.00
		010	17D	-	1.89	80.7	0.03	1.4	0.05	1.9	0.00

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
1	0 µg/day	011	22R	-	1.47	67.8	0.04	1.9	0.05	2.2	0.00
		012	22R	-	3.77	77.5	0.08	1.7	0.06	1.2	0.00
		013	22R	-	7.62	80.3	0.12	1.3	0.14	1.4	0.03
		014	22R	-	3.91	85.1	0.05	1.2	0.03	0.7	0.00
		015	22R	-	4.02	78.7	0.08	1.5	0.06	1.2	0.01
2	30 µg/day	016	4D	--	9.19	70.5	0.28	2.2	0.13	1.0	0.04
			17D	-	2.49	38.7	0.19	3.0	0.13	2.0	0.01
		017	4D	--	9.00	71.6	0.28	2.3	0.04	0.3	0.04
			17D	-	3.34	37.8	0.21	2.3	0.10	1.1	0.02
		018	4D	--	10.85	75.9	0.26	1.8	0.10	0.7	0.04
			17D	-	6.78	46.6	0.45	3.1	0.17	1.1	0.05
		019	4D	--	5.39	74.1	0.11	1.5	0.03	0.4	0.01
			17D	-	3.18	43.2	0.15	2.1	0.15	2.1	0.01
		020	4D	--	8.33	69.4	0.17	1.4	0.07	0.5	0.03
			17D	-	4.88	38.1	0.37	2.9	0.11	0.9	0.02
		021	4D	--	5.47	82.5	0.09	1.3	0.05	0.7	0.01
			17D	-	1.72	49.2	0.06	1.8	0.13	3.7	0.00

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
2	30 µg/day	022	4D	--	5.86	64.7	0.20	2.2	0.18	2.0	0.04
			17D	-	4.11	43.5	0.25	2.7	0.17	1.8	0.02
		023	17D	-	1.84	46.5	0.14	3.5	0.06	1.4	0.01
		024	17D	-	4.03	42.0	0.17	1.8	0.26	2.7	0.01
		025	17D	-	5.55	46.5	0.35	2.9	0.13	1.0	0.02
		026	22R	-	2.93	68.5	0.09	2.2	0.04	1.0	0.00
		027	22R	-	4.98	78.0	0.11	1.7	0.09	1.5	0.01
		028	22R	-	4.61	83.7	0.08	1.5	0.06	1.2	0.01
		029	22R	-	4.85	76.9	0.12	1.9	0.08	1.3	0.01
		030	22R	-	5.99	80.6	0.13	1.8	0.10	1.4	0.01
3	30 µg /day	031	4D	--	5.57	72.9	0.19	2.5	0.07	0.9	0.03
			17D	-	2.82	41.9	0.11	1.6	0.12	1.7	0.01
		032	4D	--	8.07	72.9	0.22	2.0	0.05	0.4	0.04
			17D	-	4.68	44.5	0.33	3.2	0.14	1.3	0.02
		033	4D	--	8.85	72.0	0.24	1.9	0.07	0.5	0.05
			17D	-	3.41	36.9	0.29	3.2	0.09	1.0	0.02
		034	4D	--	6.69	72.3	0.21	2.3	0.09	1.0	0.03

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
3	30 µg /day	034	17D	-	2.74	34.2	0.37	4.6	0.09	1.1	0.02
		035	4D	--	5.91	73.2	0.18	2.2	0.15	1.9	0.02
			17D	-	3.27	41.9	0.19	2.4	0.17	2.1	0.01
		036	4D	--	7.20	71.7	0.23	2.3	0.12	1.2	0.06
			17D	-	4.11	40.6	0.33	3.3	0.07	0.7	0.03
		037	4D	--	6.92	71.9	0.23	2.4	0.09	0.9	0.03
			17D	-	3.63	45.2	0.24	3.0	0.11	1.3	0.02
		038	17D	-	3.64	40.0	0.22	2.4	0.27	2.9	0.02
		039	17D	-	3.40	39.5	0.23	2.7	0.10	1.1	0.01
		040	17D	-	3.77	47.0	0.23	2.9	0.06	0.8	0.03
		041	22R	-	3.34	68.0	0.09	1.8	0.09	1.9	0.01
		042	22R	-	2.10	71.6	0.08	2.7	0.03	1.2	0.00
		043	22R	-	4.29	72.8	0.12	2.1	0.07	1.2	0.01
		044	22R	-	3.34	64.7	0.13	2.6	0.13	2.4	0.01
		045	22R	-	3.97	71.0	0.10	1.8	0.05	0.9	0.01

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %	MORPH none	POIK none	BURR none	SCHISTO none
1	0 µg/day	001	4D	--	0.3	0.06	0.7	-	-	-	-
			17D	-	0.1	0.03	0.7	Reported	-	-	-
		002	4D	--	0.1	0.04	0.7	-	-	-	-
			17D	-	0.0	0.03	0.8	Normal	-	-	-
		003	4D	--	0.1	0.03	0.4	-	-	-	-
			17D	-	- CL	- CL	- CL	- CL	-	-	-
		004	4D	--	0.2	0.05	0.6	-	-	-	-
			17D	-	0.1	0.04	0.7	Normal	-	-	-
		005	4D	--	0.3	0.06	0.7	-	-	-	-
			17D	-	0.0	0.01	0.6	Normal	-	-	-
		006	4D	--	0.3	0.04	0.6	-	-	-	-
			17D	-	0.2	0.03	0.5	Normal	-	-	-
		007	4D	--	0.3	0.04	0.5	-	-	-	-
			17D	-	0.2	0.04	0.7	-	-	-	-
		008	17D	-	0.0	0.03	0.7	-	-	-	-
		009	17D	-	0.1	0.00	0.1	-	-	-	-
		010	17D	-	0.1	0.02	0.6	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %		MORPH none	POIK none	BURR none	SCHISTO none
1	0 µg/day	011	22R	-	0.2	0.01	0.3		Reported	-	Present	-
		012	22R	-	0.1	0.02	0.4		Reported	-	Present	-
		013	22R	-	0.3	0.08	0.9		Reported	-	Present	-
		014	22R	-	0.1	0.03	0.7		Normal	-	-	-
		015	22R	-	0.1	0.03	0.5		Normal	-	-	-
2	30 µg/day	016	4D	--	0.3	0.43	3.3		-	-	-	-
			17D	-	0.2	0.34	5.3	SR	Reported	-	-	-
		017	4D	--	0.4	0.30	2.4		-	-	-	-
			17D	-	0.2	0.22	2.5		Reported	-	-	-
		018	4D	--	0.3	0.21	1.5		-	-	-	-
			17D	-	0.3	0.53	3.7		Normal	-	-	-
		019	4D	--	0.2	0.07	0.9		-	-	-	-
			17D	-	0.2	0.10	1.3		Reported	-	-	-
		020	4D	--	0.3	0.17	1.4		-	-	-	-
			17D	-	0.2	0.13	1.0		Reported	-	-	-
		021	4D	--	0.2	0.07	1.1		-	-	-	-
			17D	-	0.1	0.13	3.8		-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %		MORPH none	POIK none	BURR none	SCHISTO none
2	30 µg/day	022	4D	--	0.4	0.06	0.6		-	-	-	-
			17D	-	0.2	0.10	1.1		-	-	-	-
		023	17D	-	0.1	0.06	1.6		-	-	-	-
		024	17D	-	0.1	0.17	1.8		-	-	-	-
		025	17D	-	0.2	0.31	2.6		-	-	-	-
		026	22R	-	0.1	0.03	0.7		Reported	-	Present	-
		027	22R	-	0.2	0.05	0.7		Reported	-	Present	-
		028	22R	-	0.2	0.05	0.9		Reported	-	Present	-
		029	22R	-	0.2	0.05	0.8		Normal	-	-	-
		030	22R	-	0.2	0.06	0.8		Normal	-	-	-
3	30 µg /day	031	4D	--	0.4	0.40	5.3	FT	-	-	-	-
			17D	-	0.2	0.31	4.7		Normal	-	-	-
		032	4D	--	0.4	0.09	0.8		-	-	-	-
			17D	-	0.2	0.25	2.4		Reported	-	-	-
		033	4D	--	0.4	0.20	1.7		-	-	-	-
			17D	-	0.2	0.38	4.1		-	-	-	-
		034	4D	--	0.3	0.16	1.7		-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %		MORPH none	POIK none	BURR none	SCHISTO none
3	30 µg /day	034	17D	-	0.2	0.56	7.0	SR	Reported	-	-	-
		035	4D	--	0.3	0.15	1.8		-	-	-	-
			17D	-	0.1	0.41	5.3	SR	Reported	-	-	-
		036	4D	--	0.6	0.10	0.9		-	-	-	-
			17D	-	0.3	0.24	2.3		-	-	-	-
		037	4D	--	0.3	0.18	1.8		-	-	-	-
			17D	-	0.3	0.21	2.6		-	-	-	-
		038	17D	-	0.2	0.38	4.2		-	-	-	-
		039	17D	-	0.1	0.15	1.7		-	-	-	-
		040	17D	-	0.3	0.34	4.3		Reported	-	Present	-
		041	22R	-	0.1	0.02	0.5		Reported	-	-	-
		042	22R	-	0.0	0.02	0.6		Reported	-	Present	-
		043	22R	-	0.1	0.02	0.4		Normal	-	-	-
		044	22R	-	0.1	0.03	0.6		Normal	-	-	-
		045	22R	-	0.1	0.04	0.7		Normal	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
1	0 µg/day	001	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		002	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		003	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		004	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		005	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		006	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		007	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		008	17D	-	-	-	-	-	-	-	-
		009	17D	-	-	-	-	-	-	-	-
		010	17D	-	-	-	-	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
1	0 µg/day	011	22R	-	-	-	-	-	-	-	-
		012	22R	-	-	-	-	-	-	-	-
		013	22R	-	-	-	-	-	-	-	-
		014	22R	-	-	-	-	-	-	-	-
		015	22R	-	-	-	-	-	-	-	-
2	30 µg/day	016	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		017	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		018	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		019	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		020	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		021	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
2	30 µg/day	022	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		023	17D	-	-	-	-	-	-	-	-
		024	17D	-	-	-	-	-	-	-	-
		025	17D	-	-	-	-	-	-	-	-
		026	22R	-	-	-	-	-	-	-	-
		027	22R	-	-	-	-	-	-	-	-
		028	22R	-	-	-	-	-	-	-	-
		029	22R	-	-	-	-	-	-	-	-
		030	22R	-	-	-	-	-	-	-	-
3	30 µg /day	031	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		032	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		033	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		034	4D	--	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
3	30 µg /day	034	17D	-	-	-	-	-	-	-	-
		035	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		036	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		037	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		038	17D	-	-	-	-	-	-	-	-
		039	17D	-	-	-	-	-	-	-	-
		040	17D	-	-	-	-	-	-	-	-
		041	22R	-	-	-	-	-	-	-	-
		042	22R	-	-	-	-	-	-	-	-
		043	22R	-	-	-	-	-	-	-	-
		044	22R	-	-	-	-	-	-	-	-
		045	22R	-	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
1	0 µg/day	001	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	-	-	-
		002	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		003	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		004	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		005	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		006	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		007	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		008	17D	-	-	-	-	-	-	-	-
		009	17D	-	-	-	-	-	-	-	-
		010	17D	-	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
1	0 µg/day	011	22R	-	-	-	-	-	-	-	-
		012	22R	-	-	-	-	-	-	-	-
		013	22R	-	-	-	-	-	-	-	-
		014	22R	-	-	-	-	-	-	-	-
		015	22R	-	-	-	-	-	-	-	-
2	30 µg/day	016	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		017	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		018	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		019	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		020	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		021	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
2	30 µg/day	022	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		023	17D	-	-	-	-	-	-	-	-
		024	17D	-	-	-	-	-	-	-	-
		025	17D	-	-	-	-	-	-	-	-
		026	22R	-	-	-	-	-	-	-	-
		027	22R	-	-	-	-	-	-	-	-
		028	22R	-	-	-	-	-	-	-	-
		029	22R	-	-	-	-	-	-	-	-
		030	22R	-	-	-	-	-	-	-	-
3	30 µg /day	031	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		032	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		033	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		034	4D	--	-	-	-	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
3	30 µg /day	034	17D	-	-	-	-	-	-	-	-
		035	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		036	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		037	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		038	17D	-	-	-	-	-	-	-	-
		039	17D	-	-	-	-	-	-	-	-
		040	17D	-	-	-	-	-	-	-	-
		041	22R	-	-	-	-	-	-	-	-
		042	22R	-	-	-	-	-	-	-	-
		043	22R	-	-	-	-	-	-	-	-
		044	22R	-	-	-	-	-	-	-	-
		045	22R	-	-	-	-	-	-	-	-

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Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec		APTT sec		FIB mg/dL	
1	0 µg/day	001	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	-	CL	-	CL	-	CL
		002	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	14.0		14.8		273	
		003	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	-	QN	-	QN	-	QN
		004	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	14.8		14.9		249	
		005	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	14.4		14.7		239	
		006	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	14.6		13.3		275	
		007	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	14.9		10.5	RR	240	
		008	17D	-	-	-	-	-	14.2		15.1		259	
		009	17D	-	-	-	-	-	16.3		16.5		247	
		010	17D	-	-	-	-	-	13.9		15.5		243	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec		APTT sec		FIB mg/dL	
1	0 µg/day	011	22R	-	-	-	-	-	16.2		16.0		307	
		012	22R	-	-	-	-	-	15.8		17.0		250	
		013	22R	-	-	-	-	-	16.7		16.9		278	
		014	22R	-	-	-	-	-	14.4		15.9		225	
		015	22R	-	-	-	-	-	13.6		16.4		264	
2	30 µg/day	016	4D	--	-	-	-	-	-		-		-	
			17D	-	Present	-	-	-	15.4		16.8		618	RR
		017	4D	--	-	-	-	-	-		-		-	
			17D	-	Present	-	-	-	16.3		16.0		589	
		018	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	13.6		16.1		575	
		019	4D	--	-	-	-	-	-		-		-	
			17D	-	Present	-	-	-	-	QN	-	QN	-	QN
		020	4D	--	-	-	-	-	-		-		-	
			17D	-	Present	-	-	-	17.7		19.0		520	
		021	4D	--	-	-	-	-	-		-		-	
			17D	-	-	-	-	-	15.1		10.4	RR	611	RR

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec	APTT sec	FIB mg/dL	
2	30 µg/day	022	4D	--	-	-	-	-	-	-	-	
			17D	-	-	-	-	-	16.9	19.3	651	RR
		023	17D	-	-	-	-	-	15.1	15.8	626	RR
		024	17D	-	-	-	-	-	15.6	18.5	618	RR
		025	17D	-	-	-	-	-	15.0	16.6	562	
		026	22R	-	-	-	-	-	14.9	17.1	276	
		027	22R	-	-	-	-	-	18.5	18.4	264	
		028	22R	-	-	-	-	-	17.5	17.9	255	
		029	22R	-	-	-	-	-	17.0	18.6	240	
		030	22R	-	-	-	-	-	15.3	16.8	298	
3	30 µg /day	031	4D	--	-	-	-	-	-	-	-	
			17D	-	-	-	-	-	15.5	14.5	678	RR
		032	4D	--	-	-	-	-	-	-	-	
			17D	-	Present	-	-	-	15.3	12.8	611	RR
		033	4D	--	-	-	-	-	-	-	-	
			17D	-	-	-	-	-	16.3	18.2	589	
		034	4D	--	-	-	-	-	-	-	-	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec		APTT sec	FIB mg/dL	
3	30 µg /day	034	17D	-	Present	-	-	-	17.2		16.9	596	
		035	4D	--	-	-	-	-	-		-	-	
			17D	-	Present	-	-	-	16.2		18.1	678	RR
		036	4D	--	-	-	-	-	-		-	-	
			17D	-	-	-	-	-	16.5		18.2	582	
		037	4D	--	-	-	-	-	-		-	-	
			17D	-	-	-	-	-	17.5		18.0	618	RR
		038	17D	-	-	-	-	-	16.5		17.3	651	RR
		039	17D	-	-	-	-	-	16.8		17.0	549	
		040	17D	-	Present	-	-	-	15.7		16.8	509	
		041	22R	-	Present	-	-	-	18.5		18.2	266	
		042	22R	-	-	-	-	-	20.2	CE	17.9	258	
		043	22R	-	-	-	-	-	18.8		17.4	256	
		044	22R	-	-	-	-	-	20.1	CE	19.2	258	
		045	22R	-	-	-	-	-	15.8		17.9	282	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %
1	0 µg/day	046	4D	--	7.21	13.4	41.0	56.8	18.5	32.6	10.6
			17D	-	7.08	13.4	41.1	58.1	19.0	32.7	11.7
		047	4D	--	8.01	14.5	44.9	56.0	18.0	32.2	11.5
			17D	-	7.61	14.2	42.7	56.1	18.7	33.3	11.5
		048	4D	--	7.69	14.3	45.0	58.5	18.6	31.9	11.3
			17D	-	7.32	13.8	41.4	56.6	18.8	33.2	11.7
		049	4D	--	8.38	15.3	47.3	56.4	18.2	32.3	11.0
			17D	-	7.62	13.7	41.6	54.7	18.0	33.0	10.9
		050	4D	--	7.94	14.7	45.2	57.0	18.6	32.6	11.2
			17D	-	7.32	13.5	41.2	56.3	18.4	32.7	11.0
		051	4D	--	7.96	14.6	45.5	57.2	18.4	32.1	11.0
			17D	-	7.31	13.8	41.6	56.9	18.8	33.1	10.8
		052	4D	--	8.13	14.9	45.5	56.0	18.3	32.7	11.2
			17D	-	7.69	14.3	42.4	55.2	18.5	33.6	10.8
		053	17D	-	7.42	13.6	40.5	54.5	18.3	33.6	11.3
		054	17D	-	7.47	13.8	41.6	55.6	18.5	33.3	12.0
		055	17D	-	7.39	14.2	42.6	57.6	19.2	33.3	11.6

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL		MCH pg	MCHC g/dL	RDW %	
1	0 µg/day	056	22R	-	7.39	12.9	39.2	53.1		17.4	32.9	11.2	
		057	22R	-	7.06	14.0	40.7	-	RW	19.8	34.3	-	RW
		058	22R	-	6.97	13.5	41.0	58.8		19.4	33.0	10.8	
		059	22R	-	7.25	13.2	39.3	54.2		18.2	33.6	10.8	
		060	22R	-	7.64	14.6	43.7	57.1		19.1	33.5	10.4	
2	30 µg/day	061	4D	--	7.59	14.4	44.4	58.5		19.0	32.5	10.9	
			17D	-	6.73	12.5	38.2	56.8		18.5	32.6	12.3	
		062	4D	--	7.09	13.6	41.0	57.8		19.2	33.2	11.3	
			17D	-	6.70	12.1	38.1	56.8		18.1	31.9	13.5	
		063	4D	--	7.22	12.7	41.1	56.9		17.6	31.0	12.1	
			17D	-	6.81	11.9	37.2	54.6		17.5	32.1	13.0	
		064	4D	--	7.49	14.0	42.9	57.3		18.7	32.6	11.3	
			17D	-	7.09	12.9	39.5	55.7		18.1	32.6	12.6	
		065	4D	--	7.30	12.8	38.7	53.1		17.6	33.1	11.6	
			17D	-	6.75	12.1	36.7	54.3		17.9	33.0	15.8	
		066	4D	--	7.39	13.8	41.9	56.6		18.7	33.0	11.0	
			17D	-	6.88	12.6	37.9	55.1		18.2	33.1	12.6	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL		HGB g/dL		HCT %		MCV fL		MCH pg		MCHC g/dL		RDW %	
2	30 µg/day	067	4D	--	7.59		13.6		42.5		55.9		17.9		32.0		11.5	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	7.03		12.1		37.1		52.8		17.3		32.7		13.0	
		069	17D	-	6.65		12.5		38.9		58.5		18.8		32.2		13.5	
		070	17D	-	7.21		12.7		39.2		54.3		17.5		32.3		13.8	
		071	22R	-	7.72		13.8		42.2		54.6		17.9		32.8		13.4	
		072	22R	-	8.03		13.8		41.6		51.8		17.1		33.1		13.1	
		073	22R	-	7.73		14.0		43.0		55.7		18.1		32.5		13.0	
		074	22R	-	7.54		13.5		41.7		55.3		17.9		32.4		12.8	
		075	22R	-	8.17		14.5		43.8		53.7		17.8		33.1		12.9	
3	30 µg /day	076	4D	--	7.49		13.7		42.0		56.0		18.2		32.5		11.5	
			17D	-	6.65		11.7		35.7		53.7		17.6		32.7		13.1	
		077	4D	--	7.07		13.3		41.3		58.3		18.8		32.2		11.5	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	7.67		14.4		42.8		56.2		18.8		33.6		13.4	
			17D	-	7.11		13.2		39.0		-	RW	18.5		33.8		-	RW
		079	4D	--	7.48		13.9		43.3		57.9		18.5		32.0		11.5	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RBC 10^6/uL	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %
3	30 µg /day	079	17D	-	6.43	11.9	37.1	57.6	18.4	32.0	13.8
		080	4D	--	7.70	13.2	41.8	54.3	17.2	31.7	12.2
			17D	-	6.89	12.4	37.1	53.9	18.0	33.3	14.5
		081	4D	--	7.43	12.6	39.3	52.9	17.0	32.1	11.9
			17D	-	6.84	11.5	35.4	51.7	16.8	32.5	13.0
		082	4D	--	7.45	13.8	42.2	56.6	18.6	32.8	11.8
			17D	-	6.52	11.9	36.1	55.4	18.3	33.0	12.9
		083	17D	-	7.41	13.1	40.1	54.1	17.6	32.6	13.0
		084	17D	-	- CL	- CL	- CL	- CL	- CL	- CL	- CL
		085	17D	-	- QN	- QN	- QN	- QN	- QN	- QN	- QN
		086	22R	-	7.57	14.0	43.0	56.8	18.5	32.6	12.9
		087	22R	-	7.68	13.2	40.1	52.2	17.2	32.9	14.0
		088	22R	-	7.86	14.4	43.3	55.1	18.4	33.3	13.1
		089	22R	-	7.96	14.6	45.7	57.4	18.3	32.0	13.7
		090	22R	-	7.45	14.5	42.8	57.4	19.5	34.0	12.9

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL		MPV fL	WBC 10e3/uL	NEUT 10^3/uL	NEUT_P %
1	0 µg/day	046	4D	--	3.7	267	880		7.0	11.0	3.68	33.3
			17D	-	2.8	198	974		9.3	2.9	0.85	29.3
		047	4D	--	4.0	320	704		10.0	5.1	0.46	9.0
			17D	-	2.3	175	712		10.4	2.3	0.48	20.5
		048	4D	--	3.4	261	938		8.8	6.1	0.42	6.8
			17D	-	1.7	124	998		9.3	1.6	0.27	16.5
		049	4D	--	4.0	335	1014		8.3	5.2	0.62	12.0
			17D	-	2.2	168	980		8.9	2.9	0.51	17.5
		050	4D	--	4.6	365	1073		8.8	6.5	0.43	6.6
			17D	-	2.3	168	1050		9.2	2.2	0.55	24.3
		051	4D	--	3.4	271	953		9.2	4.2	0.37	8.8
			17D	-	2.1	154	882	SR	10.1	2.1	0.38	18.3
		052	4D	--	3.6	293	928		8.6	4.0	0.46	11.7
			17D	-	1.4	108	1019		9.2	2.0	0.28	14.2
		053	17D	-	2.4	178	929		10.0	1.7	0.36	21.5
		054	17D	-	2.5	187	701		9.5	2.1	0.23	11.3
		055	17D	-	3.1	229	824		9.1	1.8	0.18	10.1

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RETIC_P %	RETIC 10^3/uL	PLT 10^3/uL		MPV fL	WBC 10e3/uL		NEUT 10^3/uL	NEUT_P %	
1	0 µg/day	056	22R	-	2.6	192	843		9.0	1.7		0.25	14.6	
		057	22R	-	2.0	141	651		9.1	2.1		0.23	10.9	
		058	22R	-	2.7	188	803		9.2	2.4		0.33	14.0	
		059	22R	-	1.9	138	824		9.9	1.7		0.26	15.6	
		060	22R	-	1.4	107	817		8.8	3.8		0.19	4.9	SR
2	30 µg/day	061	4D	--	1.8	137	1130		8.7	8.5		2.66	31.2	
			17D	-	4.0	269	730		9.0	3.8		1.82	48.4	
		062	4D	--	1.6	113	966		8.9	8.0		2.43	30.5	
			17D	-	3.2	214	662	SR	9.5	6.2	SR	3.22	52.4	
		063	4D	--	2.3	166	910		9.1	5.2		1.24	23.9	
			17D	-	3.0	204	759		9.7	4.0		1.85	46.0	
		064	4D	--	2.5	187	1019		9.2	6.0		1.47	24.4	
			17D	-	2.9	206	699		9.5	4.9		1.74	35.4	
		065	4D	--	1.2	88	956		8.7	7.7		2.84	37.0	
			17D	-	2.8	189	871		9.3	5.6		3.12	56.2	
		066	4D	--	1.4	103	1042		9.2	11.4		3.02	26.5	
			17D	-	2.3	158	827		9.4	8.0		3.58	44.7	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RETIC_P %		RETIC 10^3/uL		PLT 10^3/uL		MPV fL		WBC 10e3/uL		NEUT 10^3/uL		NEUT_P %	
2	30 µg/day	067	4D	--	1.5		114		1004		8.6		8.1		2.48		30.5	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	3.0		211		882		9.5		6.6		2.06		31.4	
		069	17D	-	5.2		346		881		9.5		6.5		2.81		43.0	
		070	17D	-	2.8		202		691		9.2		5.7		2.02		35.6	
		071	22R	-	2.2		170		738		9.5		2.5		0.33		13.3	
		072	22R	-	2.0		161		953		8.6		1.7		0.25		14.6	
		073	22R	-	1.7		131		797		9.1		3.2		0.96		29.8	
		074	22R	-	2.2		166		905		9.1		3.4		0.47		13.8	
		075	22R	-	1.8		147		798		8.8		2.3		0.40		17.7	
3	30 µg /day	076	4D	--	1.3		97		849		9.5		7.6		2.67		35.4	
			17D	-	3.0		200		714		9.8		6.1		3.08		50.6	
		077	4D	--	2.6		184		1066		9.0		8.0		3.30		41.4	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	0.9		69		913		9.3		9.8		2.66		27.0	
			17D	-	2.5		178		682		10.2		9.0		4.07		45.0	
		079	4D	--	2.1		157		1160		8.9		7.9		2.58		32.7	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	RETIC_P %		RETIC 10^3/uL		PLT 10^3/uL		MPV fL		WBC 10e3/uL		NEUT 10^3/uL		NEUT_P %	
3	30 µg /day	079	17D	-	4.6		296		799		9.9		2.5		1.08		43.4	
		080	4D	--	1.7		131		1102	RP	7.5	RP	9.4		2.56		27.2	
			17D	-	2.4		165		944		9.9		6.1		2.78		45.3	
		081	4D	--	1.9		141		1044		8.6		7.9		2.59		32.8	
			17D	-	2.6		178		907		8.5		4.6		2.25		48.7	
		082	4D	--	2.1		156		681		10.1		9.4		3.79		40.3	
			17D	-	3.5		228		492		10.7		9.7		4.76		49.0	
		083	17D	-	2.4		178		765		9.1		6.6		2.13		32.0	
		084	17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		085	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		086	22R	-	1.7		129		729		9.0		1.4		0.30		21.7	
		087	22R	-	1.6		123		744		8.8		3.2		0.33		10.3	
		088	22R	-	1.3		102		872		9.2		1.7		0.21		12.5	
		089	22R	-	2.8		223		767		9.7		4.2		0.31		7.5	
		090	22R	-	1.4		104		798		9.2		3.1		0.24		7.8	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
1	0 µg/day	046	4D	--	6.89	62.4	0.30	2.7	0.06	0.5	0.02
			17D	-	1.91	66.3	0.07	2.5	0.04	1.5	0.00
		047	4D	--	4.48	88.0	0.05	1.0	0.07	1.3	0.01
			17D	-	1.79	76.9	0.03	1.1	0.02	1.0	0.00
		048	4D	--	5.56	91.0	0.05	0.8	0.05	0.8	0.01
			17D	-	1.31	80.0	0.03	2.1	0.02	1.2	0.00
		049	4D	--	4.41	84.7	0.07	1.3	0.06	1.2	0.01
			17D	-	2.22	76.0	0.10	3.6	0.05	1.9	0.01
		050	4D	--	5.93	91.1	0.06	1.0	0.05	0.8	0.01
			17D	-	1.60	71.2	0.05	2.0	0.04	1.9	0.00
		051	4D	--	3.75	88.8	0.05	1.1	0.04	1.0	0.00
			17D	-	1.56	75.4	0.08	3.8	0.04	1.8	0.00
		052	4D	--	3.36	84.6	0.07	1.7	0.07	1.6	0.00
			17D	-	1.66	83.2	0.03	1.4	0.01	0.7	0.00
		053	17D	-	1.22	73.6	0.05	2.7	0.02	1.4	0.00
		054	17D	-	1.74	84.7	0.04	1.9	0.03	1.7	0.00
		055	17D	-	1.50	83.7	0.08	4.3	0.02	1.1	0.00

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL	LYM_P %	MONO 10^3/uL	MONO_P %	EO 10^3/uL	EO_P %	BASO 10^3/uL
1	0 µg/day	056	22R	-	1.40	81.2	0.03	1.7	0.03	1.8	0.00
		057	22R	-	1.77	85.3	0.04	2.0	0.02	1.1	0.00
		058	22R	-	1.97	82.5	0.04	1.8	0.03	1.1	0.00
		059	22R	-	1.38	81.5	0.01	0.8	0.03	1.6	0.00
		060	22R	-	3.56	92.8	0.02	0.6	0.05	1.2	0.00
2	30 µg/day	061	4D	--	5.47	64.1	0.16	1.9	0.10	1.2	0.02
			17D	-	1.72	45.7	0.12	3.2	0.05	1.4	0.00
		062	4D	--	4.92	61.6	0.19	2.4	0.11	1.4	0.02
			17D	-	2.30	37.4	0.19	3.0	0.19	3.1	0.01
		063	4D	--	3.74	71.7	0.10	2.0	0.07	1.3	0.01
			17D	-	1.92	47.6	0.11	2.6	0.11	2.7	0.01
		064	4D	--	4.25	70.8	0.14	2.3	0.06	1.0	0.00
			17D	-	2.86	58.2	0.15	3.0	0.07	1.5	0.01
		065	4D	--	4.33	56.5	0.27	3.5	0.10	1.3	0.02
			17D	-	2.14	38.6	0.13	2.4	0.08	1.5	0.00
		066	4D	--	7.90	69.2	0.21	1.9	0.11	1.0	0.03
			17D	-	4.00	50.0	0.20	2.5	0.11	1.3	0.01

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL		LYM_P %		MONO 10^3/uL		MONO_P %		EO 10^3/uL		EO_P %		BASO 10^3/uL	
2	30 µg/day	067	4D	--	5.34		65.8		0.16		1.9		0.06		0.8		0.02	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	4.04		61.5		0.19		3.0		0.10		1.4		0.01	
		069	17D	-	3.43		52.6		0.16		2.5		0.05		0.7		0.01	
		070	17D	-	3.09		54.4		0.14		2.5		0.07		1.3		0.01	
		071	22R	-	2.07		83.4		0.04		1.6		0.03		1.3		0.00	
		072	22R	-	1.39		81.0		0.04		2.3		0.03		1.7		0.00	
		073	22R	-	2.16		67.1		0.06		2.0		0.01		0.4		0.00	
		074	22R	-	2.88		83.5		0.04		1.1		0.03		0.8		0.00	
		075	22R	-	1.75		77.5		0.06		2.6		0.04		1.6		0.00	
3	30 µg /day	076	4D	--	4.38		57.9		0.25		3.3		0.12		1.6		0.02	
			17D	-	2.61		43.0		0.12		2.0		0.10		1.7		0.01	
		077	4D	--	4.31		54.1		0.15		1.9		0.12		1.5		0.02	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	6.60		67.1		0.20		2.1		0.20		2.1		0.03	
			17D	-	4.29		47.5		0.18		2.0		0.11		1.3		0.01	
		079	4D	--	4.89		62.0		0.18		2.3		0.10		1.3		0.02	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LYM 10^3/uL		LYM_P %		MONO 10^3/uL		MONO_P %		EO 10^3/uL		EO_P %		BASO 10^3/uL	
3	30 µg /day	079	17D	-	1.14		45.8		0.09		3.7		0.03		1.4		0.00	
		080	4D	--	6.35		67.6		0.25		2.7		0.09		1.0		0.03	
			17D	-	2.91		47.5		0.21		3.4		0.14		2.3		0.01	
		081	4D	--	4.61		58.3		0.33		4.2		0.09		1.1		0.03	
			17D	-	2.03		43.9		0.12		2.6		0.08		1.7		0.01	
		082	4D	--	5.04		53.7		0.28		3.0		0.14		1.5		0.02	
			17D	-	4.29		44.2		0.26		2.7		0.15		1.5		0.02	
		083	17D	-	3.94		59.3		0.25		3.7		0.07		1.1		0.01	
		084	17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		085	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		086	22R	-	1.03		73.8		0.03		2.3		0.02		1.8		0.00	
		087	22R	-	2.79		86.4		0.06		1.8		0.02		0.7		0.00	
		088	22R	-	1.42		82.5		0.05		2.8		0.03		1.5		0.00	
		089	22R	-	3.63		87.2		0.10		2.5		0.07		1.6		0.01	
		090	22R	-	2.71		87.8		0.06		2.1		0.04		1.4		0.00	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %	MORPH none	POIK none	BURR none	SCHISTO none
1	0 µg/day	046	4D	--	0.2	0.09	0.8	-	-	-	-
			17D	-	0.0	0.01	0.5	Normal	-	-	-
		047	4D	--	0.2	0.02	0.4	-	-	-	-
			17D	-	0.1	0.01	0.4	Normal	-	-	-
		048	4D	--	0.2	0.03	0.5	-	-	-	-
			17D	-	0.0	0.00	0.2	Normal	-	-	-
		049	4D	--	0.1	0.03	0.6	-	-	-	-
			17D	-	0.2	0.02	0.8	Normal	-	-	-
		050	4D	--	0.1	0.02	0.4	-	-	-	-
			17D	-	0.0	0.01	0.4	-	-	-	-
		051	4D	--	0.1	0.01	0.3	-	-	-	-
			17D	-	0.0	0.01	0.7	Normal	-	-	-
		052	4D	--	0.1	0.01	0.4	-	-	-	-
			17D	-	0.0	0.01	0.3	-	-	-	-
		053	17D	-	0.1	0.01	0.6	-	-	-	-
		054	17D	-	0.0	0.01	0.4	-	-	-	-
		055	17D	-	0.1	0.01	0.7	-	-	-	-

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	BASO_P %	LUC 10^3/uL	LUC_P %	MORPH none	POIK none	BURR none	SCHISTO none
1	0 µg/day	056	22R	-	0.2	0.01	0.5	Normal	-	-	-
		057	22R	-	0.1	0.01	0.6	Normal	-	-	-
		058	22R	-	0.0	0.02	0.7	Normal	-	-	-
		059	22R	-	0.0	0.01	0.6	Normal	-	-	-
		060	22R	-	0.1	0.02	0.5	Reported	-	Present	-
2	30 µg/day	061	4D	--	0.2	0.13	1.5	-	-	-	-
			17D	-	0.0	0.05	1.3	Reported	-	-	-
		062	4D	--	0.2	0.32	4.0	-	-	-	-
			17D	-	0.2	0.24	3.9	Reported	-	-	-
		063	4D	--	0.2	0.05	1.0	-	-	-	-
			17D	-	0.1	0.04	0.9	Reported	-	-	-
		064	4D	--	0.1	0.08	1.3	-	-	-	-
			17D	-	0.1	0.09	1.8	Reported	-	Present	-
		065	4D	--	0.3	0.11	1.4	-	-	-	-
			17D	-	0.1	0.07	1.2	-	-	-	-
		066	4D	--	0.2	0.14	1.2	-	-	-	-
			17D	-	0.1	0.12	1.5	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	BASO_P %		LUC 10^3/uL		LUC_P %		MORPH none		POIK none		BURR none		SCHISTO none	
2	30 µg/day	067	4D	--	0.2		0.05		0.7		-		-		-		-	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	0.2		0.17		2.5		-		-		-		-	
		069	17D	-	0.1		0.07		1.1		-		-		-		-	
		070	17D	-	0.2		0.34		6.0	SR	Reported		-		-		-	
		071	22R	-	0.1		0.00		0.1		Normal		-		-		-	
		072	22R	-	0.0		0.01		0.5		Normal		-		-		-	
		073	22R	-	0.0		0.02		0.7		Normal		-		-		-	
		074	22R	-	0.1		0.02		0.6		Reported		-		Present		-	
		075	22R	-	0.2		0.01		0.5		Reported		-		Present		-	
3	30 µg /day	076	4D	--	0.3		0.12		1.6		-		-		-		-	
			17D	-	0.2		0.15		2.4		Reported		-		-		-	
		077	4D	--	0.3		0.07		0.9		-		-		-		-	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	0.3		0.14		1.4		-		-		-		-	
			17D	-	0.2		0.37		4.1		Reported		-		-		-	
		079	4D	--	0.2		0.11		1.4		-		-		-		-	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	BASO_P %		LUC 10^3/uL		LUC_P %		MORPH none		POIK none		BURR none		SCHISTO none	
3	30 µg /day	079	17D	-	0.2		0.14		5.6	SR	Reported		-		-		-	
		080	4D	--	0.3		0.11		1.2		-		-		-		-	
			17D	-	0.2		0.08		1.3		Reported		-		Present		-	
		081	4D	--	0.3		0.26		3.3		-		-		-		-	
			17D	-	0.2		0.13		2.8		-		-		-		-	
		082	4D	--	0.2		0.12		1.3		-		-		-		-	
			17D	-	0.2		0.22		2.3		Reported		-		Present		-	
		083	17D	-	0.2		0.24		3.7		-		-		-		-	
		084	17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		085	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		086	22R	-	0.1		0.00		0.3		Normal		-		-		-	
		087	22R	-	0.0		0.03		0.9		Normal		-		-		-	
		088	22R	-	0.3		0.01		0.4		Normal		-		-		-	
		089	22R	-	0.2		0.04		0.9		Reported		-		Present		-	
		090	22R	-	0.1		0.03		0.9		Reported		-		Present		-	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
1	0 µg/day	046	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		047	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		048	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		049	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		050	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		051	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		052	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		053	17D	-	-	-	-	-	-	-	-
		054	17D	-	-	-	-	-	-	-	-
		055	17D	-	-	-	-	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	SPHERO none	SIDERO none	TARGET none	TEAR none	B_STIP none	HJ none	AGGL none
1	0 µg/day	056	22R	-	-	-	-	-	-	-	-
		057	22R	-	-	-	-	-	-	-	-
		058	22R	-	-	-	-	-	-	-	-
		059	22R	-	-	-	-	-	-	-	-
		060	22R	-	-	-	-	-	-	-	-
2	30 µg/day	061	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		062	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		063	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		064	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		065	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		066	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	SPHERO none		SIDERO none		TARGET none		TEAR none		B_STIP none		HJ none		AGGL none	
2	30 µg/day	067	4D	--	-		-		-		-		-		-		-	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	-		-		-		-		-		-		-	
		069	17D	-	-		-		-		-		-		-		-	
		070	17D	-	-		-		-		-		-		-		-	
		071	22R	-	-		-		-		-		-		-		-	
		072	22R	-	-		-		-		-		-		-		-	
		073	22R	-	-		-		-		-		-		-		-	
		074	22R	-	-		-		-		-		-		-		-	
		075	22R	-	-		-		-		-		-		-		-	
3	30 µg /day	076	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		077	4D	--	-		-		-		-		-		-		-	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		079	4D	--	-		-		-		-		-		-		-	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	SPHERO none		SIDERO none		TARGET none		TEAR none		B_STIP none		HJ none		AGGL none	
3	30 µg /day	079	17D	-	-		-		-		-		-		-		-	
		080	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		081	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		082	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		083	17D	-	-		-		-		-		-		-		-	
		084	17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		085	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		086	22R	-	-		-		-		-		-		-		-	
		087	22R	-	-		-		-		-		-		-		-	
		088	22R	-	-		-		-		-		-		-		-	
		089	22R	-	-		-		-		-		-		-		-	
		090	22R	-	-		-		-		-		-		-		-	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
1	0 µg/day	046	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		047	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		048	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		049	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		050	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		051	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		052	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		053	17D	-	-	-	-	-	-	-	-
		054	17D	-	-	-	-	-	-	-	-
		055	17D	-	-	-	-	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	CLPLT none	HGB_CRYS none	BASOPH none	ACANTH none	STOM none	OTHERM none	DOHLE none
1	0 µg/day	056	22R	-	-	-	-	-	-	-	-
		057	22R	-	-	-	-	-	-	-	-
		058	22R	-	-	-	-	-	-	-	-
		059	22R	-	-	-	-	-	-	-	-
		060	22R	-	-	-	-	-	-	-	-
2	30 µg/day	061	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		062	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	-	-	-
		063	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		064	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		065	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-
		066	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	-	-	-

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	CLPLT none		HGB_CRYS none		BASOPH none		ACANTH none		STOM none		OTHERM none		DOHLE none	
2	30 µg/day	067	4D	--	-		-		-		-		-		-		-	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	17D	-	-		-		-		-		-		-		-	
		069	17D	-	-		-		-		-		-		-		-	
		070	17D	-	-		-		-		-		-		-		-	
		071	22R	-	-		-		-		-		-		-		-	
		072	22R	-	-		-		-		-		-		-		-	
		073	22R	-	-		-		-		-		-		-		-	
		074	22R	-	-		-		-		-		-		-		-	
		075	22R	-	-		-		-		-		-		-		-	
3	30 µg /day	076	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		077	4D	--	-		-		-		-		-		-		-	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		078	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		079	4D	--	-		-		-		-		-		-		-	

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	CLPLT none		HGB_CRYS none		BASOPH none		ACANTH none		STOM none		OTHERM none		DOHLE none	
3	30 µg /day	079	17D	-	-		-		-		-		-		-		-	
		080	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		081	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		082	4D	--	-		-		-		-		-		-		-	
			17D	-	-		-		-		-		-		-		-	
		083	17D	-	-		-		-		-		-		-		-	
		084	17D	-	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL	-	CL
		085	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		086	22R	-	-		-		-		-		-		-		-	
		087	22R	-	-		-		-		-		-		-		-	
		088	22R	-	-		-		-		-		-		-		-	
		089	22R	-	-		-		-		-		-		-		-	
		090	22R	-	-		-		-		-		-		-		-	

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Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec	APTT sec	FIB mg/dL
1	0 µg/day	046	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	14.4	15.6	215
		047	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	15.1	16.4	219
		048	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	14.7	16.3	201
		049	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	13.5	15.9	235
		050	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	13.4	14.2	263
		051	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	13.5	15.0	204
		052	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	14.6	16.0	220
		053	17D	-	-	-	-	-	14.6	15.9	188
		054	17D	-	-	-	-	-	14.9	14.9	183
		055	17D	-	-	-	-	-	12.5	14.3	244

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Hematology and Coagulation
20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	HYSEG none	TOXIC_G none	TOXIC_V none	VACLYM none	PT_Rat sec	APTT sec	FIB mg/dL
1	0 µg/day	056	22R	-	-	-	-	-	14.3	16.2	189
		057	22R	-	-	-	-	-	12.0	15.7	185
		058	22R	-	-	-	-	-	13.3	17.3	173
		059	22R	-	-	-	-	-	13.0	17.8	171
		060	22R	-	-	-	-	-	12.9	17.1	214
2	30 µg/day	061	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	15.2	12.7	520
		062	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	13.7	15.2	549
		063	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	14.2	16.1	462
		064	4D	--	-	-	-	-	-	-	-
			17D	-	Present	-	-	-	17.0	14.3	509
		065	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	14.8	15.7	526
		066	4D	--	-	-	-	-	-	-	-
			17D	-	-	-	-	-	14.6	17.0	526

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Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	HYSEG none		TOXIC_G none		TOXIC_V none		VACLYM none		PT_Rat sec		APTT sec		FIB mg/dL	
2	30 µg/day	067	4D	--	-		-		-		-		-		-		-	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	QN	-	QN	-	QN
		068	17D	-	-		-		-		-		14.5		15.7		504	
		069	17D	-	-		-		-		-		15.9		16.0		603	RR
		070	17D	-	Present		-		-		-		14.1		17.3		678	RR
		071	22R	-	-		-		-		-		13.8		17.0		165	
		072	22R	-	-		-		-		-		13.5		16.7		209	
		073	22R	-	-		-		-		-		13.9		16.4		210	
		074	22R	-	-		-		-		-		14.7		17.5		197	
		075	22R	-	-		-		-		-		12.4		18.7		202	
3	30 µg /day	076	4D	--	-		-		-		-		-		-		-	
			17D	-	Present		-		-		-		14.9		16.7		643	RR
		077	4D	--	-		-		-		-		-		-		-	
			17D	-	-	CL	-	CL	-	CL	-	CL	-	QN	-	QN	-	QN
		078	4D	--	-		-		-		-		-		-		-	
			17D	-	Present		-		-		-		15.6		16.6		603	RR
		079	4D	--	-		-		-		-		-		-		-	

Appendix 7

Hematology and Coagulation

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	HYSEG none		TOXIC_G none		TOXIC_V none		VACLYM none		PT_Rat sec	APTT sec		FIB mg/dL	
3	30 µg /day	079	17D	-	Present		-		-		-		16.3	16.6		634	RR
		080	4D	--	-		-		-		-		-	-		-	
			17D	-	Present		-		-		-		14.3	16.2		531	
		081	4D	--	-		-		-		-		-	-		-	
			17D	-	-		-		-		-		15.2	15.2		520	
		082	4D	--	-		-		-		-		-	-		-	
			17D	-	Present		-		-		-		15.6	15.9		499	
		083	17D	-	-		-		-		-		15.3	16.9		603	RR
		084	17D	-	-	CL	-	CL	-	CL	-	CL	17.1	8.8	RR	515	
		085	17D	-	-	QN	-	QN	-	QN	-	QN	14.1	10.1	RR	520	
		086	22R	-	-		-		-		-		12.9	16.2		188	
		087	22R	-	-		-		-		-		14.0	17.2		160	
		088	22R	-	-		-		-		-		14.0	17.7		182	
		089	22R	-	-		-		-		-		14.1	16.2		189	
		090	22R	-	-		-		-		-		12.9	17.5		206	

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
GGT	Gamma Glutamyl Transferase
TBIL	Bilirubin, Total
CHOL	Cholesterol
TRIG	Triglycerides
GLUC	Glucose
TP	Protein, Total
ALB	Albumin
GLOB	Globulin
AG	Albumin/Globulin Ratio
BUN	Blood Urea Nitrogen
CREA	Creatinine
PHOS	Phosphorus
CA	Calcium
NA	Sodium
K	Potassium
CL	Chloride
HEM_IND	Hemolytic Index

Parameter	Description
ICT_IND	Icterus Index
LIP_IND	Lipemic Index
A2M	Alpha-2-Macroglobulin
A1AGP	Alpha-1 Acid Glycoprotein

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Comment	Description
NS	No Sample
QN	Quantity Not Sufficient
RR	Result repeated

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

- Value not applicable;

Note: Each interval/day will be concatenated with the phase name abbreviation

HPD = Hours Post Dose; U = Unscheduled

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
1	0 µg/day	001	17D	-	22	63	92	<3	<0.1	46	69
		002	17D	-	18	72	88	<3	<0.1	50	87
		003	17D	-	17	71	79	<3	0.1	62	42
		004	17D	-	20	71	85	<3	<0.1	54	64
		005	17D	-	19	70	145	<3	<0.1	54	64
		006	17D	-	17	76	86	<3	<0.1	44	56
		007	17D	-	20	75	146	<3	<0.1	46	73
		008	4D	---	34	88	130	<3	<0.1	57	35
			17D	-	15	75	74	<3	<0.1	44	30
		009	4D	---	27	99	135	<3	<0.1	60	76
			17D	-	19	80	88	<3	<0.1	57	45
		010	4D	---	32	108	179	<3	<0.1	78	103
			17D	-	14	64	93	<3	<0.1	60	58
		011	4D	---	28	84	173	<3	<0.1	54	59
			22R	-	25	108	85	<3	<0.1	49	42
		012	4D	---	16	96	171	<3	<0.1	61	33
			22R	-	17	89	93	<3	<0.1	56	80

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
1	0 µg/day	013	4D	---	27	102	131	<3	<0.1	52	44
			22R	-	18	95	74	<3	<0.1	44	35
		014	4D	---	40	86	134	<3	<0.1	72	91
			22R	-	18	82	63	<3	<0.1	52	37
		015	4D	---	29	93	280	<3	<0.1	70	55
			22R	-	18	85	107	<3	<0.1	63	51
2	30 µg/day	016	17D	-	20	92	93	<3	0.1	37	44
		017	17D	-	25	94	126	<3	<0.1	33	38
		018	17D	-	24	84	94	<3	0.1	42	44
		019	17D	-	20	63	105	<3	<0.1	44	38
		020	17D	-	24	105	91	<3	<0.1	40	31
		021	17D	-	16	68	114	<3	<0.1	41	22
		022	17D	-	18	63	67	<3	<0.1	28	28
		023	4D	---	36	104	199	<3	<0.1	47	42
			17D	-	25	103	132	<3	<0.1	48	30
		024	4D	---	47	122	193	<3	<0.1	53	32
			17D	-	33	89	100	<3	<0.1	43	28

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
2	30 µg/day	025	4D	---	32	91	214	<3	<0.1	66	44
			17D	-	24	81	112	<3	<0.1	46	33
		026	4D	---	33	126	182	<3	<0.1	58	52
			22R	-	17	103	81	<3	<0.1	68	77
		027	4D	---	29	108	201	<3	<0.1	43	31
			22R	-	22	105	75	<3	<0.1	56	46
		028	4D	---	25	97	136	<3	<0.1	55	34
			22R	-	16	102	71	<3	<0.1	66	40
		029	4D	---	34	87	206	<3	<0.1	49	60
			22R	-	17	86	76	<3	<0.1	59	42
		030	4D	---	30	90	232	<3	<0.1	49	47
			22R	-	16	74	95	<3	<0.1	56	49
3	30 µg /day	031	17D	-	18	76	97	<3	<0.1	32	29
		032	17D	-	22	72	103	<3	<0.1	30	38
		033	17D	-	21	88	101	<3	<0.1	35	59
		034	17D	-	19	91	95	<3	<0.1	44	28
		035	17D	-	24	94	152	<3	0.1	46	38

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
3	30 µg /day	036	17D	-	19	81	80	<3	<0.1	28	28
		037	17D	-	26	88	113	<3	<0.1	32	31
		038	4D	---	24	94	206	<3	<0.1	39	27
			17D	-	20	94	146	<3	<0.1	38	24
		039	4D	---	32	110	202	<3	<0.1	78	70
			17D	-	16	99	97	<3	<0.1	55	44
		040	4D	---	29	81	216	<3	<0.1	46	61
			17D	-	23	85	116	<3	<0.1	32	40
		041	4D	---	41	82	186	<3	<0.1	58	62
			22R	-	22	93	94	<3	<0.1	54	71
		042	4D	---	27	93	177	<3	<0.1	45	71
			22R	-	16	95	68	<3	<0.1	54	48
		043	4D	---	26	123	143	<3	<0.1	41	26
			22R	-	14	105	66	<3	<0.1	54	38
		044	4D	---	24	97	153	<3	<0.1	37	33
			22R	-	18	96	79	<3	<0.1	55	28
		045	4D	---	27	102	223	<3	<0.1	70	65

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
3	30 µg /day	045	22R	-	17	96	110	<3	<0.1	64	43

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
1	0 µg/day	001	17D	-	148	5.7	3.7	2.0	1.8	22	0.3
		002	17D	-	138	5.3	3.4	1.9	1.8	22	0.3
		003	17D	-	136	5.2	3.4	1.8	1.9	18	0.3
		004	17D	-	100	5.2	3.4	1.8	1.9	12	0.2
		005	17D	-	137	5.8	3.8	2.0	1.9	24	0.2
		006	17D	-	138	5.4	3.5	1.9	1.8	16	0.3
		007	17D	-	104	5.8	3.7	2.1	1.8	15	0.2
		008	4D	---	98	5.9	3.8	2.1	1.8	26	0.3
			17D	-	152	5.0	3.3	1.7	1.9	23	0.3
		009	4D	---	100	5.9	3.9	2.0	2.0	20	0.3
			17D	-	128	5.5	3.6	1.9	1.9	18	0.2
		010	4D	---	129	6.2	4.0	2.2	1.8	28	0.3
			17D	-	136	5.0	3.2	1.8	1.8	18	0.2
		011	4D	---	109	5.8	3.8	2.0	1.9	19	0.3
			22R	-	110	5.9	3.8	2.1	1.8	20	0.3
		012	4D	---	124	6.4	4.2	2.2	1.9	29	0.4
			22R	-	157	5.6	3.6	2.0	1.8	16	0.3

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
1	0 µg/day	013	4D	---	90	6.1	4.0	2.1	1.9	18	0.3
			22R	-	117	5.9	3.8	2.1	1.8	17	0.3
		014	4D	---	117	6.3	4.1	2.2	1.9	20	0.3
			22R	-	121	6.0	3.8	2.2	1.7	16	0.2
		015	4D	---	123	6.2	4.0	2.2	1.8	30	0.3
			22R	-	180	5.7	3.6	2.1	1.7	16	0.3
2	30 µg/day	016	17D	-	143	5.9	3.6	2.3	1.6	23	0.4
		017	17D	-	100	5.8	3.6	2.2	1.6	16	0.2
		018	17D	-	142	5.3	3.4	1.9	1.8	24	0.3
		019	17D	-	129	5.9	3.7	2.2	1.7	16	0.3
		020	17D	-	102	5.0	3.1	1.9	1.6	14	0.2
		021	17D	-	114	5.7	3.4	2.3	1.5	19	0.2
		022	17D	-	125	5.1	3.2	1.9	1.7	20	0.2
		023	4D	---	100	5.6	3.6	2.0	1.8	27	0.3
			17D	-	103	5.9	3.7	2.2	1.7	18	0.3
		024	4D	---	79	6.1	3.9	2.2	1.8	24	0.3
			17D	-	118	5.2	3.3	1.9	1.7	16	0.2

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
2	30 µg/day	025	4D	---	89	6.0	3.8	2.2	1.7	22	0.3
			17D	-	98	5.3	3.3	2.0	1.6	20	0.2
		026	4D	---	97	5.8	3.6	2.2	1.6	25	0.2
			22R	-	149	6.2	3.9	2.3	1.7	18	0.3
		027	4D	---	116	5.9	3.7	2.2	1.7	35	0.3
			22R	-	138	6.1	3.8	2.3	1.7	17	0.3
		028	4D	---	115	5.6	3.5	2.1	1.7	25	0.3
			22R	-	98	5.9	3.7	2.2	1.7	16	0.3
		029	4D	---	98	6.2	3.9	2.3	1.7	23	0.3
			22R	-	96	6.1	3.9	2.2	1.8	19	0.2
		030	4D	---	91	6.0	3.7	2.3	1.6	27	0.3
			22R	-	126	6.1	3.8	2.3	1.7	16	0.3
3	30 µg /day	031	17D	-	124	5.1	3.1	2.0	1.6	25	0.3
		032	17D	-	119	5.2	3.3	1.9	1.7	19	0.3
		033	17D	-	160	6.0	3.8	2.2	1.7	22	0.3
		034	17D	-	139	5.3	3.3	2.0	1.6	22	0.3
		035	17D	-	100	6.0	3.7	2.3	1.6	21	0.3

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
3	30 µg /day	036	17D	-	158	5.2	3.3	1.9	1.7	17	0.2
		037	17D	-	120	5.6	3.5	2.1	1.7	18	0.3
		038	4D	---	78	5.9	3.8	2.1	1.8	22	0.2
			17D	-	98	5.3	3.3	2.0	1.6	21	0.3
		039	4D	---	91	6.1	3.8	2.3	1.7	25	0.2
			17D	-	118	5.3	3.3	2.0	1.6	18	0.2
		040	4D	---	110	5.7	3.6	2.1	1.7	22	0.3
			17D	-	90	5.1	3.2	1.9	1.7	16	0.2
		041	4D	---	101	5.9	3.7	2.2	1.7	20	0.3
			22R	-	142	6.0	3.8	2.2	1.7	20	0.3
		042	4D	---	131	6.0	3.8	2.2	1.7	25	0.3
			22R	-	124	5.8	3.7	2.1	1.8	12	0.3
		043	4D	---	85	5.4	3.4	2.0	1.7	23	0.2
			22R	-	117	5.7	3.5	2.2	1.6	17	0.2
		044	4D	---	109	5.8	3.6	2.2	1.6	29	0.3
			22R	-	119	6.0	3.8	2.2	1.7	13	0.3
		045	4D	---	95	6.0	3.7	2.3	1.6	24	0.3

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
3	30 µg /day	045	22R	-	97	6.0	3.8	2.2	1.7	20	0.3

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL		CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
1	0 µg/day	001	17D	-	8.8		9.8	146	4.2	106	Negative	Negative
		002	17D	-	8.3		9.7	145	4.2	105	Slight	Negative
		003	17D	-	8.6		10.0	143	4.6	105	Negative	Negative
		004	17D	-	7.7		9.7	142	4.2	104	Negative	Negative
		005	17D	-	10.2		10.2	143	4.5	103	Negative	Negative
		006	17D	-	8.3		9.9	143	4.3	105	Negative	Negative
		007	17D	-	8.9		10.4	145	4.3	105	Negative	Negative
		008	4D	---	7.1		9.5	144	4.2	102	Negative	Negative
			17D	-	9.7		9.9	144	4.2	105	Slight	Negative
		009	4D	---	7.3		9.4	143	4.1	101	Negative	Negative
			17D	-	8.7		9.9	145	4.1	104	Negative	Negative
		010	4D	---	7.6		9.7	144	5.0	100	Negative	Negative
			17D	-	8.0		9.1	144	4.4	106	Negative	Negative
		011	4D	---	7.3		10.0	144	4.7	102	Slight	Negative
			22R	-	4.7	RR	9.4	142	4.2	106	Negative	Negative
		012	4D	---	6.8		9.9	148	4.6	109	Slight	Negative
			22R	-	6.2		9.3	142	4.4	106	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL	CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
1	0 µg/day	013	4D	---	6.6	9.6	144	4.3	102	Slight	Negative
			22R	-	7.2	9.5	143	4.0	106	Slight	Negative
		014	4D	---	7.6	10.1	145	4.2	102	Negative	Negative
			22R	-	7.2	9.6	143	4.3	107	Slight	Negative
		015	4D	---	8.4	9.9	143	4.5	101	Slight	Negative
			22R	-	7.5	9.4	142	3.7	103	Negative	Negative
2	30 µg/day	016	17D	-	7.2	9.8	141	4.4	102	Negative	Negative
		017	17D	-	8.0	10.1	142	4.4	101	Negative	Negative
		018	17D	-	7.6	9.3	138	4.1	101	Negative	Negative
		019	17D	-	7.5	10.2	144	4.4	105	Negative	Negative
		020	17D	-	8.2	9.2	141	4.4	104	Negative	Negative
		021	17D	-	8.2	9.9	142	4.5	102	Slight	Negative
		022	17D	-	8.6	9.7	143	5.0	106	Negative	Negative
		023	4D	---	7.1	9.3	145	4.4	104	Negative	Negative
			17D	-	8.2	10.2	144	4.4	105	Negative	Negative
		024	4D	---	7.7	9.8	145	4.5	102	Negative	Negative
			17D	-	9.2	9.8	144	4.2	105	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL	CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
2	30 µg/day	025	4D	---	7.1	9.9	144	4.3	102	Negative	Negative
			17D	-	8.4	10.0	144	3.8	103	Negative	Negative
		026	4D	---	7.9	9.7	143	4.8	101	Negative	Negative
			22R	-	6.4	9.9	142	4.4	105	Negative	Negative
		027	4D	---	7.3	9.4	142	4.5	101	Negative	Negative
			22R	-	6.9	9.4	142	4.6	107	Slight	Negative
		028	4D	---	6.7	9.3	143	4.5	102	Negative	Negative
			22R	-	7.6	9.2	143	4.0	107	Negative	Negative
		029	4D	---	7.5	9.8	146	4.6	102	Negative	Negative
			22R	-	6.8	9.2	144	4.3	105	Negative	Negative
		030	4D	---	8.0	10.0	145	4.8	102	Negative	Negative
			22R	-	6.6	9.5	142	4.0	106	Negative	Negative
3	30 µg /day	031	17D	-	7.6	9.2	140	4.2	105	Slight	Negative
		032	17D	-	6.5	9.4	143	4.1	106	Negative	Negative
		033	17D	-	6.7	10.0	143	4.6	103	Negative	Negative
		034	17D	-	8.5	9.3	139	4.2	102	Negative	Negative
		035	17D	-	7.9	10.0	143	4.3	104	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL	CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
3	30 µg /day	036	17D	-	8.4	9.6	143	4.2	104	Negative	Negative
		037	17D	-	9.6	9.7	142	4.7	103	Slight	Negative
		038	4D	---	7.5	9.4	143	4.5	102	Negative	Negative
			17D	-	8.2	9.4	145	4.3	106	Slight	Negative
		039	4D	---	7.8	10.1	145	4.6	99	Negative	Negative
			17D	-	8.7	9.9	144	4.2	104	Negative	Negative
		040	4D	---	7.1	9.7	145	4.2	101	Negative	Negative
			17D	-	8.0	9.4	145	4.4	105	Negative	Negative
		041	4D	---	8.1	10.0	143	4.7	100	Negative	Negative
			22R	-	6.8	9.3	144	4.1	106	Negative	Negative
		042	4D	---	7.0	9.7	144	5.0	101	Negative	Negative
			22R	-	5.6	9.2	144	3.9	108	Negative	Negative
		043	4D	---	7.5	9.5	142	4.7	103	Negative	Negative
			22R	-	7.0	9.5	143	4.4	107	Slight	Negative
		044	4D	---	7.9	9.4	143	4.5	102	Negative	Negative
			22R	-	7.4	9.5	142	4.3	105	Negative	Negative
		045	4D	---	7.7	10.2	145	5.1	102	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL	CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
3	30 µg /day	045	22R	-	7.3	9.9	144	4.3	106	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
1	0 µg/day	001	17D	-	Negative	12	68.67
		002	17D	-	Negative	15	52.23
		003	17D	-	Negative	19	24.30
		004	17D	-	Negative	12	34.45
		005	17D	-	Negative	11	43.22
		006	17D	-	Negative	9	60.45
		007	17D	-	Negative	15	53.83
		008	4D	---	Negative	679	948.10
			17D	-	Negative	13	47.23
		009	4D	---	Negative	53	75.58
			17D	-	Negative	19	50.35
		010	4D	---	Negative	44	63.18
			17D	-	Negative	15	41.99
		011	4D	---	Negative	37	75.39
			22R	-	Negative	10	91.41
		012	4D	---	Negative	32	61.74
			22R	-	Negative	9	43.29

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
1	0 µg/day	013	4D	---	Negative	21	48.94
			22R	-	Negative	9	49.54
		014	4D	---	Negative	17	55.88
			22R	-	Negative	6	43.79
		015	4D	---	Negative	24	66.05
			22R	-	Negative	6	46.52
2	30 µg/day	016	17D	-	Negative	1801	2632.51
		017	17D	-	Negative	886	1974.42
		018	17D	-	Negative	576	1817.56
		019	17D	-	Negative	223	1176.32
		020	17D	-	Negative	783	1720.69
		021	17D	-	Negative	2686	2072.64
		022	17D	-	Negative	724	1782.38
		023	4D	---	Negative	1325	1356.32
			17D	-	Negative	395	1845.24
		024	4D	---	Negative	2333	1571.10
			17D	-	Negative	997	1794.51

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
2	30 µg/day	025	4D	---	Negative	1139	1636.32
			17D	-	Negative	835	1543.59
		026	4D	---	Negative	2678	2378.00
			22R	-	Negative	9	66.75
		027	4D	---	Negative	1679	1616.32
			22R	-	Negative	10	69.36
		028	4D	---	Negative	2817	1627.92
			22R	-	Negative	16	55.29
		029	4D	---	Negative	3963	1499.70
			22R	-	Negative	44	65.90
		030	4D	---	Negative	2611	1452.44
			22R	-	Negative	18	121.40
3	30 µg /day	031	17D	-	Negative	2132	2019.27
		032	17D	-	Negative	2439	1495.94
		033	17D	-	Negative	1104	2068.21
		034	17D	-	Negative	1496	1355.49
		035	17D	-	Negative	4973	3485.55

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
3	30 µg /day	036	17D	-	Negative	1321	1953.14
		037	17D	-	Negative	1688	2785.50
		038	4D	---	Negative	1370	1538.05
			17D	-	Negative	858	2072.35
		039	4D	---	Negative	4147	1841.85
			17D	-	Negative	1072	1311.89
		040	4D	---	Negative	3404	2437.64
			17D	-	Negative	859	1663.49
		041	4D	---	Negative	1457	1815.29
			22R	-	Negative	15	84.77
		042	4D	---	Negative	10485	4831.95
			22R	-	Negative	19	55.24
		043	4D	---	Negative	4106	2495.36
			22R	-	Negative	17	46.84
		044	4D	---	Negative	2755	1830.75
			22R	-	Negative	13	50.71
		045	4D	---	Negative	3569	2023.44

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
3	30 µg /day	045	22R	-	Negative	17	75.25

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	ALT U/L		AST U/L		ALP U/L		GGT U/L		TBIL mg/dL		CHOL mg/dL		TRIG mg/dL	
1	0 µg/day	046	17D	-	12		88		60		<3		<0.1		39		45	
		047	17D	-	9		66		62		<3		<0.1		28		32	
		048	17D	-	12		70		37		<3		<0.1		20		20	
		049	17D	-	11		52		56		<3		<0.1		48		29	
		050	17D	-	11		102		65		<3		<0.1		26		22	
		051	17D	-	11		46		48		<3		0.1		28		22	
		052	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		053	4D	---	26		74		65		<3		<0.1		33		29	
			17D	-	14		75		38		<3		0.1		27		19	
		054	4D	---	28		94		96		<3		<0.1		41		32	
			17D	-	11		78		48		<3		<0.1		30		34	
		055	4D	---	18		64		91		<3		<0.1		72		62	
			17D	-	11		52		44		<3		<0.1		55		27	
		056	4D	---	15		99		94		<3		<0.1		34		32	
			22R	-	10		77		36		3		<0.1		26		22	
		057	4D	---	21		87		84		<3		<0.1		43		25	
			22R	-	15		59		34		<3		0.1		56		40	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	ALT U/L		AST U/L		ALP U/L		GGT U/L		TBIL mg/dL		CHOL mg/dL		TRIG mg/dL	
1	0 µg/day	058	4D	---	20		78		131		<3		<0.1		53		31	
			22R	-	13		61		41		<3		0.1		53		35	
		059	4D	---	21		74		113		<3		<0.1		54		52	
			22R	-	11		59		35		<3		<0.1		47		36	
		060	4D	---	18		84		69		<3		<0.1		35		31	
			22R	-	10		71		24		<3		<0.1		33		21	
2	30 µg/day	061	17D	-	14		96		73		<3		<0.1		41		30	
		062	17D	-	13		88		76		<3		<0.1		33		35	
		063	17D	-	13		71		87		<3		<0.1		34		25	
		064	17D	-	13		81		110		<3		<0.1		24		20	
		065	17D	-	15		96		89		<3		0.1		28		21	
		066	17D	-	10		57		49		<3		<0.1		32		22	
		067	17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	4D	---	29		110		132		<3		<0.1		32		24	
			17D	-	18		103		58		<3		<0.1		29		21	
		069	4D	---	23		90		123		<3		<0.1		47		24	
			17D	-	14		81		80		<3		0.1		42		29	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	ALT U/L		AST U/L		ALP U/L		GGT U/L		TBIL mg/dL		CHOL mg/dL		TRIG mg/dL	
2	30 µg/day	070	4D	---	27		79		108		<3		<0.1		63		34	
			17D	-	15		62		81		<3		<0.1		40		23	
		071	4D	---	20		84		134		<3		<0.1		41		26	
			22R	-	14		62		35		<3		0.1		49		33	
		072	4D	---	30		120		182		<3		<0.1		68		43	
			22R	-	17		82		46		<3		<0.1		75		37	
		073	4D	---	20		98		144		<3		<0.1		39		33	
			22R	-	12		86		41		<3		<0.1		34		30	
		074	4D	---	21		83		135		<3		<0.1		41		24	
			22R	-	14		72		31		<3		<0.1		40		32	
		075	4D	---	29		105		145		<3		<0.1		47		27	
			22R	-	17		66		32		<3		<0.1		73		27	
3	30 µg /day	076	17D	-	19		113		118		<3		0.1		33		28	
		077	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		078	17D	-	15		66		123		<3		0.1		32		22	
		079	17D	-	22		86		87		<3		<0.1		31		29	
		080	17D	-	13		87		94		<3		<0.1		34		36	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	ALT U/L		AST U/L		ALP U/L		GGT U/L		TBIL mg/dL		CHOL mg/dL		TRIG mg/dL	
3	30 µg /day	081	17D	-	12		52		80		<3		<0.1		23		23	
		082	17D	-	16		71		107		<3		<0.1		30		29	
		083	4D	---	20		85		163		<3		<0.1		71		31	
			17D	-	17		85		102		<3		<0.1		36		25	
		084	4D	---	33		109		157		<3		<0.1		57		46	
			17D	-	18		82		68		<3		<0.1		36		20	
		085	4D	---	21		93		181		<3		<0.1		57		28	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		086	4D	---	29		103		163		<3		<0.1		50		31	
			22R	-	16		70		29		<3		<0.1		44		27	
		087	4D	---	18		93		157		<3		<0.1		39		28	
			22R	-	14		62		37		<3		<0.1		29		37	
		088	4D	---	21		79		125		<3		<0.1		47		28	
			22R	-	13		69		23		<3		<0.1		37		32	
		089	4D	---	23		83		110		<3		<0.1		77		38	
			22R	-	14		72		36		<3		<0.1		52		45	
		090	4D	---	20		85		91		<3		<0.1		55		46	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	ALT U/L	AST U/L	ALP U/L	GGT U/L	TBIL mg/dL	CHOL mg/dL	TRIG mg/dL
3	30 µg /day	090	22R	-	11	63	20	<3	<0.1	44	45

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL		TP g/dL		ALB g/dL		GLOB g/dL		AG none		BUN mg/dL		CREA mg/dL	
1	0 µg/day	046	17D	-	118		4.8		3.3		1.5		2.2		17		0.2	
		047	17D	-	128		5.5		3.7		1.8		2.1		17		0.3	
		048	17D	-	105		5.5		3.5		2.0		1.8		14		0.2	
		049	17D	-	97		5.5		3.6		1.9		1.9		23		0.3	
		050	17D	-	97		5.3		3.5		1.8		1.9		13		0.2	
		051	17D	-	127		5.6		3.7		1.9		1.9		16		0.3	
		052	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		053	4D	---	112		6.6		4.4		2.2		2.0		15		0.3	
			17D	-	83		6.0		4.0		2.0		2.0		15		0.3	
		054	4D	---	94		6.4		4.2		2.2		1.9		17		0.3	
			17D	-	122		5.3		3.5		1.8		1.9		19		0.2	
		055	4D	---	115		6.7		4.5		2.2		2.0		20		0.4	
			17D	-	126		5.5		3.6		1.9		1.9		19		0.4	
		056	4D	---	90		5.6		3.8		1.8		2.1		17		0.3	
			22R	-	108		6.2		4.0		2.2		1.8		17		0.4	
		057	4D	---	101		6.0		4.0		2.0		2.0		19		0.3	
			22R	-	130		6.5		4.4		2.1		2.1		13		0.3	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL		TP g/dL		ALB g/dL		GLOB g/dL		AG none		BUN mg/dL		CREA mg/dL	
1	0 µg/day	058	4D	---	101		6.3		4.2		2.1		2.0		15		0.3	
			22R	-	129		7.1		4.7		2.4		2.0		17		0.4	
		059	4D	---	101		6.4		4.2		2.2		1.9		15		0.3	
			22R	-	130		6.6		4.3		2.3		1.9		21		0.4	
		060	4D	---	106		6.1		4.0		2.1		1.9		16		0.3	
			22R	-	100		6.2		3.9		2.3		1.7		15		0.3	
2	30 µg/day	061	17D	-	90		5.4		3.3		2.1		1.6		20		0.2	
		062	17D	-	111		5.1		3.1		2.0		1.6		20		0.3	
		063	17D	-	98		4.9		3.1		1.8		1.7		23		0.3	
		064	17D	-	106		5.0		3.1		1.9		1.6		22		0.2	
		065	17D	-	89		5.0		3.0		2.0		1.5		16		0.2	
		066	17D	-	107		4.9		3.0		1.9		1.6		14		0.2	
		067	17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	4D	---	72		5.7		3.6		2.1		1.7		16		0.2	
			17D	-	94		4.9		3.0		1.9		1.6		22		0.2	
		069	4D	---	92		5.7		3.6		2.1		1.7		14		0.2	
			17D	-	102		4.6		2.9		1.7		1.7		15		0.2	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
2	30 µg/day	070	4D	---	94	5.9	3.7	2.2	1.7	25	0.3
			17D	-	100	5.0	3.1	1.9	1.6	18	0.2
		071	4D	---	89	5.7	3.6	2.1	1.7	16	0.2
			22R	-	115	6.8	4.3	2.5	1.7	14	0.3
		072	4D	---	97	5.6	3.5	2.1	1.7	20	0.2
			22R	-	98	6.5	4.1	2.4	1.7	18	0.3
		073	4D	---	97	5.3	3.4	1.9	1.8	17	0.2
			22R	-	96	6.3	4.0	2.3	1.7	20	0.3
		074	4D	---	85	5.6	3.5	2.1	1.7	25	0.2
			22R	-	121	6.7	4.2	2.5	1.7	21	0.3
		075	4D	---	87	5.7	3.6	2.1	1.7	17	0.3
			22R	-	108	6.4	4.1	2.3	1.8	19	0.3
3	30 µg /day	076	17D	-	106	5.4	3.3	2.1	1.6	19	0.3
		077	17D	-	- QN	- QN	- QN	- QN	- QN	- QN	- QN
		078	17D	-	90	5.2	3.2	2.0	1.6	21	0.2
		079	17D	-	107	5.0	3.1	1.9	1.6	20	0.2
		080	17D	-	88	5.0	3.2	1.8	1.8	20	0.2

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL		TP g/dL		ALB g/dL		GLOB g/dL		AG none		BUN mg/dL		CREA mg/dL	
3	30 µg /day	081	17D	-	103		4.9		3.0		1.9		1.6		19		0.2	
		082	17D	-	89		4.4		2.9		1.5		1.9		21		0.2	
		083	4D	---	85		5.8		3.6		2.2		1.6		19		0.2	
			17D	-	105		4.9		3.0		1.9		1.6		18		0.2	
		084	4D	---	91		5.7		3.6		2.1		1.7		23		0.2	
			17D	-	108		4.9		3.0		1.9		1.6		22		0.2	
		085	4D	---	88		6.2		3.9		2.3		1.7		19		0.3	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		086	4D	---	75		5.7		3.6		2.1		1.7		14		0.2	
			22R	-	108		6.3		4.1		2.2		1.9		17		0.3	
		087	4D	---	96		5.7		3.6		2.1		1.7		17		0.3	
			22R	-	104		6.8		4.3		2.5		1.7		20		0.3	
		088	4D	---	87		6.1		3.8		2.3		1.7		19		0.3	
			22R	-	95		6.9		4.4		2.5		1.8		20		0.3	
		089	4D	---	87		6.2		3.9		2.3		1.7		18		0.3	
			22R	-	137		7.1		4.6		2.5		1.8		18		0.4	
		090	4D	---	88		6.1		3.8		2.3		1.7		17		0.2	

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	GLUC mg/dL	TP g/dL	ALB g/dL	GLOB g/dL	AG none	BUN mg/dL	CREA mg/dL
3	30 µg /day	090	22R	-	146	6.6	4.2	2.4	1.8	16	0.3

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL		CA mg/dL		NA mmol/L		K mmol/L		CL mmol/L		HEM_IND none		ICT_IND none	
1	0 µg/day	046	17D	-	8.2		9.4		144		4.7		109		Negative		Negative	
		047	17D	-	8.4		9.4		143		4.0		107		Negative		Negative	
		048	17D	-	7.1		9.5		145		4.3		108		Negative		Negative	
		049	17D	-	7.2		9.4		145		4.4		108		Negative		Negative	
		050	17D	-	7.4		9.5		144		4.8		109		Negative		Negative	
		051	17D	-	6.0		9.5		142		4.3		109		Negative		Negative	
		052	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		053	4D	---	6.2		9.8		144		3.7		104		Negative		Negative	
			17D	-	5.9		9.5		144		4.2		108		Negative		Negative	
		054	4D	---	7.8		9.9		144		3.7		104		Slight		Negative	
			17D	-	8.5		9.7		143		4.6		106		Negative		Negative	
		055	4D	---	6.9		10.2		144		3.9		105		Negative		Negative	
			17D	-	7.6		9.8		142		4.8		108		Negative		Negative	
		056	4D	---	6.3		9.4		142		4.0		103		Negative		Negative	
			22R	-	6.1		9.5		143		3.6		107		Negative		Negative	
		057	4D	---	6.6		9.5		143		3.8		103		Negative		Negative	
			22R	-	7.2		10.1		142		4.0		107		Negative		Negative	

Appendix 8

Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL		CA mg/dL		NA mmol/L		K mmol/L		CL mmol/L		HEM_IND none		ICT_IND none	
1	0 µg/day	058	4D	---	6.4		9.6		144		3.7		107		Slight		Negative	
			22R	-	7.0		9.9		144		3.8		108		Negative		Negative	
		059	4D	---	6.0		9.7		144		4.0		104		Negative		Negative	
			22R	-	5.3		9.4		139		3.5		103		Negative		Negative	
		060	4D	---	6.7		9.5		145		4.0		103		Slight		Negative	
			22R	-	6.8		9.9		143		4.3		109		Negative		Negative	
2	30 µg/day	061	17D	-	7.4		9.8		144		4.8		106		Negative		Negative	
		062	17D	-	8.1		9.9		144		4.3		110		Slight		Negative	
		063	17D	-	7.0		9.3		144		4.5		109		Negative		Negative	
		064	17D	-	8.3		9.6		142		4.4		106		Negative		Negative	
		065	17D	-	7.3		9.7		141		4.6		106		Negative		Negative	
		066	17D	-	6.7		9.7		143		4.3		110		Negative		Negative	
		067	17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		068	4D	---	7.0		9.5		143		4.4		104		Slight		Negative	
			17D	-	7.2		9.1		145		4.7		109		Negative		Negative	
		069	4D	---	6.0		9.4		144		3.9		107		Negative		Negative	
			17D	-	7.6		9.4		142		4.5		107		Negative		Negative	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL		CA mg/dL		NA mmol/L		K mmol/L		CL mmol/L		HEM_IND none		ICT_IND none	
2	30 µg/day	070	4D	---	6.7		9.5		143		4.6		105		Negative		Negative	
			17D	-	6.8		9.3		142		4.7		106		Negative		Negative	
		071	4D	---	7.0		9.8		142		4.2		105		Negative		Negative	
			22R	-	5.6		9.7		143		3.9		107		Negative		Negative	
		072	4D	---	7.3		9.5		142		4.6		101		Negative		Negative	
			22R	-	5.4		9.7		142		4.2		104		Slight		Negative	
		073	4D	---	5.9		9.4		143		4.2		104		Negative		Negative	
			22R	-	6.3		9.8		144		4.1		106		Negative		Negative	
		074	4D	---	7.2		9.8		143		4.9		104		Negative		Negative	
			22R	-	7.6		10.0		143		4.0		107		Negative		Negative	
		075	4D	---	7.4		9.8		145		3.8		106		Negative		Negative	
			22R	-	6.6		9.8		144		3.8		106		Negative		Negative	
3	30 µg /day	076	17D	-	7.2		9.5		143		4.6		109		Negative		Negative	
		077	17D	-	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN	-	QN
		078	17D	-	7.8		9.9		143		4.4		108		Negative		Negative	
		079	17D	-	9.7		10.0		143		5.2		107		Negative		Negative	
		080	17D	-	6.6		9.6		142		4.7		108		Negative		Negative	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL		CA mg/dL		NA mmol/L		K mmol/L		CL mmol/L		HEM_IND none		ICT_IND none	
3	30 µg /day	081	17D	-	6.9		9.6		144		4.6		110		Negative		Negative	
		082	17D	-	8.0		9.2		144		4.8		109		Negative		Negative	
		083	4D	---	6.7		10.0		142		4.4		105		Negative		Negative	
			17D	-	8.6		9.9		142		4.9		106		Slight		Negative	
		084	4D	---	6.9		10.1		144		4.8		105		Negative		Negative	
			17D	-	7.0		9.5		144		4.8		108		Slight		Negative	
		085	4D	---	6.2		9.7		146		4.2		107		Negative		Negative	
			17D	-	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS	-	NS
		086	4D	---	6.6		9.3		143		4.1		103		Negative		Negative	
			22R	-	7.8		9.5		144		4.0		107		Negative		Negative	
		087	4D	---	8.1		9.5		145		3.8		108		Negative		Negative	
			22R	-	7.0		9.8		144		3.7		106		Negative		Negative	
		088	4D	---	6.9		10.1		144		4.7		107		Negative		Negative	
			22R	-	6.1		9.9		143		4.1		108		Negative		Negative	
		089	4D	---	6.6		9.9		142		4.8		103		Negative		Negative	
			22R	-	7.4		10.3		142		3.9		106		Negative		Negative	
		090	4D	---	7.3		9.9		144		4.3		103		Slight		Negative	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	PHOS mg/dL	CA mg/dL	NA mmol/L	K mmol/L	CL mmol/L	HEM_IND none	ICT_IND none
3	30 µg /day	090	22R	-	5.5	9.6	141	4.3	107	Negative	Negative

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LIP_IND none		A2M ug/mL	A1AGP ug/mL
1	0 µg/day	046	17D	-	Negative		33	94.42
		047	17D	-	Negative		22	80.84
		048	17D	-	Negative		173	328.40
		049	17D	-	Negative		15	71.66
		050	17D	-	Negative		10	80.68
		051	17D	-	Negative		26	69.19
		052	17D	-	-	QN	13	49.83
		053	4D	---	Negative		211	287.21
			17D	-	Negative		18	54.31
		054	4D	---	Negative		530	391.89
			17D	-	Negative		11	67.97
		055	4D	---	Negative		612	582.86
			17D	-	Negative		10	62.29
		056	4D	---	Negative		22	72.80
			22R	-	Negative		16	84.10
		057	4D	---	Negative		20	165.20
			22R	-	Negative		11	54.10

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LIP_IND none		A2M ug/mL		A1AGP ug/mL	
1	0 µg/day	058	4D	---	Negative		255		220.09	
			22R	-	Negative		32		48.05	
		059	4D	---	Negative		29		77.14	
			22R	-	Negative		15		45.77	
		060	4D	---	Negative		18		121.00	
			22R	-	Negative		12		81.92	
2	30 µg/day	061	17D	-	Negative		948		1837.15	
		062	17D	-	Negative		599		1641.31	
		063	17D	-	Negative		249		1335.38	
		064	17D	-	Negative		796		1360.18	
		065	17D	-	Negative		405		1320.56	
		066	17D	-	Negative		260		1397.00	
		067	17D	-	-	NS	-	NS	-	NS
		068	4D	---	Negative		1214		1990.76	
			17D	-	Negative		484		1043.02	
		069	4D	---	Negative		255		1426.67	
			17D	-	Negative		231		1358.38	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LIP_IND none		A2M ug/mL		A1AGP ug/mL	
2	30 µg/day	070	4D	---	Negative		511		1993.04	
			17D	-	Negative		717		2133.66	
		071	4D	---	Negative		966		1716.82	
			22R	-	Negative		14		26.72	
		072	4D	---	Negative		1231		2125.32	
			22R	-	Negative		15		53.78	
		073	4D	---	Negative		289		1471.09	
			22R	-	Negative		11		46.76	
		074	4D	---	Negative		744		1933.38	
			22R	-	Negative		26		53.28	
		075	4D	---	Negative		420		2593.43	
			22R	-	Negative		15		59.02	
3	30 µg /day	076	17D	-	Negative		989		2110.38	
		077	17D	-	-	QN	-	QN	-	QN
		078	17D	-	Negative		953		1992.92	
		079	17D	-	Negative		530		1837.66	
		080	17D	-	Negative		399		1607.53	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LIP_IND none		A2M ug/mL		A1AGP ug/mL	
3	30 µg /day	081	17D	-	Negative		447		1295.34	
		082	17D	-	Negative		588		855.25	
		083	4D	---	Negative		1178		2088.91	
			17D	-	Negative		377		1754.23	
		084	4D	---	Negative		597		1882.91	
			17D	-	Negative		453		1755.26	
		085	4D	---	Negative		886		1606.16	
			17D	-	-	NS	-	NS	-	NS
		086	4D	---	Negative		883		1965.13	
			22R	-	Negative		16		39.63	
		087	4D	---	Negative		563		1350.77	
			22R	-	Negative		22		73.06	
		088	4D	---	Negative		606		1429.46	
			22R	-	Negative		18		34.16	
		089	4D	---	Negative		1600		1484.34	
			22R	-	Negative		13		63.68	
		090	4D	---	Negative		784		1609.14	

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Clinical Chemistry

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	HPD	LIP_IND none	A2M ug/mL	A1AGP ug/mL
3	30 µg /day	090	22R	-	Negative	11	77.41

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Parameter	Description
COLOR	Color
CLARITY	Clarity
pH	pH
GLU	Glucose Urine
KET	Ketones
PRO	Protein
BIL	Bilirubin
BLOOD	Blood
SG	Specific Gravity
VOLUME	Total Volume
F_ELEM	Formed Elements
U_RBC	RBC, Urine
U_WBC	WBC, Urine
SQ_EPI	Epithelial Cells, Squamous
TR_PHOS	Crystal, Triple Phosphate
SPERM	Sperm

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

- Value not applicable;

Note: Each interval/day will be concatenated with the phase name abbreviation

HPD = Hours Post Dose; U = Unscheduled

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
1	0 µg/day	001	17D	Dark Yellow	Clear	7.5	Negative	15	30	Negative	Negative
		002	17D	Dark Yellow	Clear	6.5	Negative	15	30	Negative	Negative
		003	17D	Dark Yellow	Cloudy	7.0	Negative	15	30	Negative	Negative
		004	17D	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		005	17D	Dark Yellow	Clear	6.5	Negative	15	30	Negative	Negative
		006	17D	Yellow	Clear	7.5	Negative	Trace	Negative	Negative	Negative
		007	17D	Yellow	Clear	7.5	Negative	Negative	Negative	Negative	Negative
		008	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		009	17D	Dark Yellow	Cloudy	7.0	Negative	15	100	Negative	Negative
		010	17D	Dark Yellow	Clear	7.5	Negative	Trace	30	Negative	Negative
		011	22R	Dark Yellow	Cloudy	7.0	Negative	15	30	Negative	Negative
		012	22R	Dark Yellow	Clear	7.0	Negative	15	30	Negative	Negative
		013	22R	Dark Yellow	Clear	7.5	Negative	15	30	Negative	Negative
		014	22R	Dark Yellow	Clear	7.0	Negative	15	30	Negative	Negative
		015	22R	Dark Yellow	Clear	8.0	Negative	15	30	Negative	Negative
2	30 µg/day	016	17D	Yellow	Clear	6.5	Negative	Trace	Trace	Negative	Negative
		017	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		018	17D	Dark Yellow	Cloudy	6.5	Negative	Trace	100	Negative	Negative
		019	17D	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		020	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative

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Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
2	30 µg/day	021	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		022	17D	Dark Yellow	Cloudy	6.0	Negative	15	100	Small	Negative
		023	17D	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		024	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		025	17D	Yellow	Clear	7.0	Negative	Trace	Negative	Negative	Negative
		026	22R	Dark Yellow	Clear	7.0	Negative	15	30	Negative	Negative
		027	22R	Yellow	Clear	7.5	Negative	Trace	Trace	Negative	Negative
		028	22R	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		029	22R	Dark Yellow	Clear	7.5	Negative	15	30	Negative	Negative
		030	22R	Yellow	Clear	7.0	Negative	15	30	Negative	Negative
3	30 µg /day	031	17D	Dark Yellow	Clear	6.5	Negative	15	30	Negative	Negative
		032	17D	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		033	17D	Dark Yellow	Cloudy	6.0	Negative	Trace	100	Small	Negative
		034	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		035	17D	Yellow	Clear	6.5	Negative	Trace	30	Negative	Negative
		036	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		037	17D	Yellow	Clear	6.5	Negative	Trace	Trace	Negative	Negative
		038	17D	Dark Yellow	Clear	6.5	Negative	Trace	30	Negative	Negative
		039	17D	Yellow	Clear	6.5	Negative	Trace	Negative	Negative	Negative
		040	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative

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Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
3	30 µg /day	041	22R	Dark Yellow	Cloudy	7.0	Negative	15	30	Negative	Negative
		042	22R	Yellow	Clear	7.5	Negative	Negative	Negative	Negative	Negative
		043	22R	Dark Yellow	Clear	7.0	Negative	40	100	Negative	Negative
		044	22R	Dark Yellow	Clear	6.5	Negative	15	30	Negative	Negative
		045	22R	Yellow	Clear	7.0	Negative	Trace	30	Negative	Negative

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Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF	SPERM none
1	0 µg/day	001	17D	1.035	7.0	Reported	-	-	-	Many	Present
		002	17D	1.049	5.0	Reported	-	-	0-5	-	Present
		003	17D	1.043	6.0	Reported	-	0-5	0-5	Moderate	Present
		004	17D	1.016	16.0	Reported	-	-	0-5	-	Present
		005	17D	1.047	5.0	Reported	-	-	0-5	-	Present
		006	17D	1.013	17.0	-	-	-	-	-	-
		007	17D	1.008	37.0	-	-	-	-	-	-
		008	17D	1.006	48.0	-	-	-	-	-	-
		009	17D	1.067	2.0	-	-	-	-	-	-
		010	17D	1.038	6.0	-	-	-	-	-	-
		011	22R	1.051	3.0	Reported	-	-	0-5	Moderate	Present
		012	22R	1.059	4.5	Reported	-	0-5	0-5	Few	Present
		013	22R	1.057	3.0	Reported	0-5	-	0-5	Few	Present
		014	22R	1.059	3.0	Reported	-	-	0-5	Few	Present
		015	22R	1.052	5.0	Reported	-	0-5	0-5	Moderate	Present
2	30 µg/day	016	17D	1.020	13.0	Reported	-	0-5	0-5	Few	Present
		017	17D	1.013	17.0	Reported	-	-	0-5	-	Present
		018	17D	1.063	3.0	Reported	-	-	0-5	Many	-
		019	17D	1.029	9.0	Reported	-	-	-	Few	Present
		020	17D	1.007	28.0	Reported	-	-	0-5	-	Present

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF	SPERM none
2	30 µg/day	021	17D	1.005	60.0	-	-	-	-	-	-
		022	17D	1.070	2.0	-	-	-	-	-	-
		023	17D	1.025	8.0	-	-	-	-	-	-
		024	17D	1.011	23.0	-	-	-	-	-	-
		025	17D	1.017	15.0	-	-	-	-	-	-
		026	22R	1.048	4.0	Reported	0-5	-	-	Few	Present
		027	22R	1.026	10.0	Reported	-	0-5	0-5	Few	Present
		028	22R	1.013	17.0	Reported	-	0-5	0-5	-	Present
		029	22R	1.046	4.0	Reported	-	0-5	0-5	Moderate	Present
		030	22R	1.037	6.0	Reported	0-5	0-5	0-5	Few	Present
3	30 µg /day	031	17D	1.056	3.0	Reported	-	-	0-5	Few	Present
		032	17D	1.023	9.0	Reported	-	0-5	0-5	Few	Present
		033	17D	1.060	3.0	Reported	-	0-5	0-5	Few	Present
		034	17D	1.013	20.0	Reported	-	-	0-5	-	Present
		035	17D	1.022	9.0	Reported	-	-	0-5	Few	Present
		036	17D	1.016	17.0	-	-	-	-	-	-
		037	17D	1.020	13.0	-	-	-	-	-	-
		038	17D	1.045	5.0	-	-	-	-	-	-
		039	17D	1.014	15.0	-	-	-	-	-	-
		040	17D	1.013	22.0	-	-	-	-	-	-

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF	SPERM none
3	30 µg /day	041	22R	1.057	3.0	Reported	-	0-5	0-5	Moderate	Present
		042	22R	1.011	27.0	Reported	-	0-5	0-5	-	Present
		043	22R	1.070	2.0	Reported	-	0-5	0-5	Few	Present
		044	22R	1.052	3.0	Reported	-	0-5	0-5	Moderate	Present
		045	22R	1.030	5.0	Reported	-	-	0-5	Few	Present

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
1	0 µg/day	046	17D	Dark Yellow	Clear	6.5	Negative	Trace	Negative	Negative	Negative
		047	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		048	17D	Dark Yellow	Clear	6.5	Negative	Negative	Trace	Negative	Negative
		049	17D	Yellow	Clear	7.0	Negative	Negative	Trace	Negative	Negative
		050	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		051	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		052	17D	Dark Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		053	17D	Dark Yellow	Clear	6.5	Negative	Trace	Trace	Negative	Negative
		054	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		055	17D	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		056	22R	Yellow	Clear	7.0	Negative	Trace	Trace	Negative	Negative
		057	22R	Yellow	Clear	7.0	Negative	Negative	Negative	Negative	Negative
		058	22R	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		059	22R	Dark Yellow	Clear	6.5	Negative	Trace	Trace	Negative	Negative
		060	22R	Yellow	Clear	8.0	Negative	Negative	Negative	Negative	Negative
2	30 µg/day	061	17D	Dark Yellow	Clear	6.0	Negative	Negative	Trace	Negative	Negative
		062	17D	Dark Yellow	Clear	6.0	Negative	Trace	Trace	Negative	Negative
		063	17D	Dark Yellow	Clear	6.0	Negative	Trace	30	Negative	Negative
		064	17D	Yellow	Clear	6.0	Negative	Negative	Negative	Negative	Negative
		065	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
2	30 µg/day	066	17D	Dark Yellow	Clear	6.5	Negative	Negative	Trace	Negative	Negative
		067	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		068	17D	Dark Yellow	Clear	6.0	Negative	Negative	Trace	Negative	Negative
		069	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		070	17D	Dark Yellow	Cloudy	6.0	Negative	Negative	Trace	Negative	Negative
		071	22R	Dark Yellow	Clear	6.5	Negative	Trace	Trace	Negative	Negative
		072	22R	Yellow	Clear	6.0	Negative	Negative	Negative	Negative	Negative
		073	22R	Dark Yellow	Clear	7.0	Negative	15	30	Negative	Negative
		074	22R	Yellow	Clear	7.5	Negative	Negative	Negative	Negative	Negative
		075	22R	Dark Yellow	Clear	6.0	Negative	Trace	Trace	Negative	Negative
3	30 µg /day	076	17D	Dark Yellow	Clear	6.5	Negative	Negative	Trace	Negative	Negative
		077	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		078	17D	Dark Yellow	Clear	6.5	Negative	Trace	30	Negative	Negative
		079	17D	Yellow	Clear	6.0	Negative	Trace	Trace	Negative	Negative
		080	17D	Yellow	Clear	6.0	Negative	Negative	Negative	Negative	Negative
		081	17D	Dark Yellow	Cloudy	6.0	Negative	Trace	30	Negative	Negative
		082	17D	Yellow	Clear	6.0	Negative	Negative	Negative	Negative	Negative
		083	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		084	17D	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		085	17D	Dark Yellow	Clear	5.5	Negative	Negative	30	Negative	Negative

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	COLOR none	CLARITY none	pH none	GLU mg/dL	KET mg/dL	PRO mg/dL	BIL none	BLOOD none
3	30 µg /day	086	22R	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		087	22R	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative
		088	22R	Dark Yellow	Clear	7.0	Negative	Trace	30	Negative	Negative
		089	22R	Dark Yellow	Clear	6.0	Negative	Negative	Trace	Negative	Negative
		090	22R	Yellow	Clear	6.5	Negative	Negative	Negative	Negative	Negative

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF
1	0 µg/day	046	17D	1.035	5.0	Reported	-	0-5	0-5	Few
		047	17D	1.024	8.0	Reported	-	-	0-5	Few
		048	17D	1.035	4.0	Reported	-	-	0-5	-
		049	17D	1.033	5.0	Reported	-	0-5	0-5	Few
		050	17D	1.010	17.0	Reported	-	-	0-5	-
		051	17D	1.015	12.0	-	-	-	-	-
		052	17D	1.029	5.0	-	-	-	-	-
		053	17D	1.044	4.0	-	-	-	-	-
		054	17D	1.011	14.0	-	-	-	-	-
		055	17D	1.007	25.0	-	-	-	-	-
		056	22R	1.042	3.0	Reported	-	0-5	0-5	-
		057	22R	1.011	18.0	Reported	0-5	0-5	0-5	-
		058	22R	1.010	15.0	Reported	-	0-5	0-5	-
		059	22R	1.044	3.0	Reported	0-5	0-5	0-5	Few
		060	22R	1.013	16.0	Reported	-	0-5	0-5	-
2	30 µg/day	061	17D	1.046	4.0	Reported	-	-	0-5	-
		062	17D	1.037	2.0	Reported	-	0-5	0-5	-
		063	17D	1.054	2.0	Reported	-	-	0-5	Few
		064	17D	1.008	26.0	Reported	-	-	0-5	-
		065	17D	1.014	12.0	Reported	-	0-5	0-5	-

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF
2	30 µg/day	066	17D	1.036	4.0	-	-	-	-	-
		067	17D	1.011	17.0	-	-	-	-	-
		068	17D	1.034	4.0	-	-	-	-	-
		069	17D	1.011	22.0	-	-	-	-	-
		070	17D	1.037	3.0	-	-	-	-	-
		071	22R	1.044	3.5	Reported	0-5	0-5	0-5	-
		072	22R	1.016	14.0	Reported	-	0-5	0-5	-
		073	22R	1.059	1.5	Reported	-	0-5	0-5	Few
		074	22R	1.021	8.0	Reported	-	0-5	0-5	Few
		075	22R	1.042	3.0	Reported	-	0-5	0-5	-
3	30 µg /day	076	17D	1.033	3.0	Reported	-	-	0-5	Few
		077	17D	1.011	20.0	Reported	-	0-5	0-5	-
		078	17D	1.035	4.0	Reported	-	0-5	0-5	-
		079	17D	1.028	5.0	Reported	-	0-5	0-5	-
		080	17D	1.010	16.0	Reported	-	-	0-5	-
		081	17D	1.038	3.0	-	-	-	-	-
		082	17D	1.025	7.0	-	-	-	-	-
		083	17D	1.011	15.0	-	-	-	-	-
		084	17D	1.010	18.0	-	-	-	-	-
		085	17D	1.049	3.0	-	-	-	-	-

Appendix 9

Urinalysis

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal	Day	SG none	VOLUME mL	F_ELEM none	U_RBC /HPF	U_WBC /HPF	SQ_EPI /LPF	TR_PHOS /HPF
3	30 µg /day	086	22R	1.009	20.0	Reported	-	0-5	0-5	-
		087	22R	1.021	8.0	Reported	-	0-5	0-5	Few
		088	22R	1.055	1.5	Reported	-	0-5	0-5	Few
		089	22R	1.041	3.0	Reported	-	0-5	0-5	-
		090	22R	1.012	12.5	Reported	-	0-5	0-5	-

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

- = Value not applicable; NW = Not Weighed; e = Excluded.
ABS = Absolute Value; OW = Organ Weight; BW = Body Weight; BRN = Brain Weight;
OW:BW = (g/g)*100; OW:BRN = g/g.

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Epididymis			Gland, Adrenal		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	001	Dosing-Terminal Euthanasia	269.7	1.834	0.680	1.000	1.242	0.461	0.677	0.070	0.026	0.038
		002	Dosing-Terminal Euthanasia	321.3	1.931	0.601	1.000	1.522	0.474	0.788	0.083	0.026	0.043
		003	Dosing-Terminal Euthanasia	300.0	1.951	0.650	1.000	1.275	0.425	0.654	0.077	0.026	0.039
		004	Dosing-Terminal Euthanasia	315.7	2.098	0.665	1.000	1.224	0.388	0.583	0.063	0.020	0.030
		005	Dosing-Terminal Euthanasia	294.1	1.928	0.656	1.000	1.211	0.412	0.628	0.071	0.024	0.037
		006	Dosing-Terminal Euthanasia	304.8	1.851	0.607	1.000	1.018	0.334	0.550	0.066	0.022	0.036
		007	Dosing-Terminal Euthanasia	304.1	1.894	0.623	1.000	0.907	0.298	0.479	0.073	0.024	0.039
		008	Dosing-Terminal Euthanasia	286.7	1.760	0.614	1.000	1.136	0.396	0.645	0.070	0.024	0.040
		009	Dosing-Terminal Euthanasia	276.4	1.946	0.704	1.000	1.066	0.386	0.548	0.062	0.022	0.032
		010	Dosing-Terminal Euthanasia	287.8	1.868	0.649	1.000	1.046	0.363	0.560	0.062	0.022	0.033
		011	Recovery-Recovery Euthanasia 1	329.10	2.173	0.660	1.000	1.454	0.442	0.669	0.065	0.020	0.030
		012	Recovery-Recovery Euthanasia 1	362.80	2.080	0.573	1.000	1.522	0.420	0.732	0.062	0.017	0.030
		013	Recovery-Recovery Euthanasia 1	304.00	2.098	0.690	1.000	1.216	0.400	0.580	0.072	0.024	0.034
		014	Recovery-Recovery Euthanasia 1	283.80	2.052	0.723	1.000	1.341	0.473	0.654	0.053	0.019	0.026
		015	Recovery-Recovery Euthanasia 1	379.20	2.133	0.563	1.000	1.268	0.334	0.594	0.083	0.022	0.039

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Epididymis			Gland, Adrenal		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	016	Dosing-Terminal Euthanasia	257.4	1.765	0.686	1.000	1.055	0.410	0.598	0.072	0.028	0.041
		017	Dosing-Terminal Euthanasia	284.4	1.949	0.685	1.000	0.885	0.311	0.454	0.066	0.023	0.034
		018	Dosing-Terminal Euthanasia	267.3	1.839	0.688	1.000	0.878	0.328	0.477	0.061	0.023	0.033
		019	Dosing-Terminal Euthanasia	289.1	1.772	0.613	1.000	1.129	0.391	0.637	0.099	0.034	0.056
		020	Dosing-Terminal Euthanasia	250.2	2.006	0.802	1.000	1.105	0.442	0.551	0.062	0.025	0.031
		021	Dosing-Terminal Euthanasia	269.6	2.062	0.765	1.000	1.058	0.392	0.513	0.068	0.025	0.033
		022	Dosing-Terminal Euthanasia	268.5	1.746	0.650	1.000	1.162	0.433	0.666	0.064	0.024	0.037
		023	Dosing-Terminal Euthanasia	274.5	1.830	0.667	1.000	1.119	0.408	0.611	0.095	0.035	0.052
		024	Dosing-Terminal Euthanasia	248.5	2.033	0.818	1.000	0.949	0.382	0.467	0.056	0.023	0.028
		025	Dosing-Terminal Euthanasia	302.2	2.157	0.714	1.000	1.286	0.426	0.596	0.084	0.028	0.039
		026	Recovery-Recovery Euthanasia 1	351.50	1.815	0.516	1.000	1.131	0.322	0.623	0.068	0.019	0.037
		027	Recovery-Recovery Euthanasia 1	340.00	1.818	0.535	1.000	1.023	0.301	0.563	0.078	0.023	0.043
		028	Recovery-Recovery Euthanasia 1	335.40	2.080	0.620	1.000	1.310	0.391	0.630	0.115	0.034	0.055
		029	Recovery-Recovery Euthanasia 1	350.40	2.062	0.588	1.000	1.259	0.359	0.611	0.101	0.029	0.049
		030	Recovery-Recovery Euthanasia 1	380.20	2.020	0.531	1.000	1.550	0.408	0.767	0.090	0.024	0.045

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Epididymis			Gland, Adrenal		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	031	Dosing-Terminal Euthanasia	244.7	1.808	0.739	1.000	1.116	0.456	0.617	0.075	0.031	0.041
		032	Dosing-Terminal Euthanasia	254.6	1.897	0.745	1.000	1.083	0.425	0.571	0.052	0.020	0.027
		033	Dosing-Terminal Euthanasia	301.1	1.945	0.646	1.000	0.970	0.322	0.499	0.077	0.026	0.040
		034	Dosing-Terminal Euthanasia	250.4	1.842	0.736	1.000	1.087	0.434	0.590	0.074	0.030	0.040
		035	Dosing-Terminal Euthanasia	235.8	1.922	0.815	1.000	1.018	0.432	0.530	0.081	0.034	0.042
		036	Dosing-Terminal Euthanasia	278.8	1.898	0.681	1.000	1.025	0.368	0.540	0.085	0.030	0.045
		037	Dosing-Terminal Euthanasia	273.2	1.861	0.681	1.000	0.953	0.349	0.512	0.077	0.028	0.041
		038	Dosing-Terminal Euthanasia	258.7	1.951	0.754	1.000	1.006	0.389	0.516	0.062	0.024	0.032
		039	Dosing-Terminal Euthanasia	262.2	1.947	0.743	1.000	1.159	0.442	0.595	0.064	0.024	0.033
		040	Dosing-Terminal Euthanasia	266.4	2.011	0.755	1.000	1.091	0.410	0.543	0.059	0.022	0.029
		041	Recovery-Recovery Euthanasia 1	339.00	1.922	0.567	1.000	1.394	0.411	0.725	0.069	0.020	0.036
		042	Recovery-Recovery Euthanasia 1	325.50	1.962	0.603	1.000	1.433	0.440	0.730	0.063	0.019	0.032
		043	Recovery-Recovery Euthanasia 1	344.20	1.939	0.563	1.000	1.401	0.407	0.723	0.070	0.020	0.036
		044	Recovery-Recovery Euthanasia 1	347.50	1.938	0.558	1.000	1.472	0.424	0.760	0.083	0.024	0.043
		045	Recovery-Recovery Euthanasia 1	315.60	1.943	0.616	1.000	1.302	0.413	0.670	0.099	0.031	0.051

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Gland, Prostate			Heart			Kidney		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	001	Dosing-Terminal Euthanasia	269.7	0.704	0.261	0.384	0.946	0.351	0.516	1.935	0.717	1.055
		002	Dosing-Terminal Euthanasia	321.3	0.834	0.260	0.432	0.900	0.280	0.466	2.224	0.692	1.152
		003	Dosing-Terminal Euthanasia	300.0	0.799	0.266	0.410	0.925	0.308	0.474	2.439	0.813	1.250
		004	Dosing-Terminal Euthanasia	315.7	0.867	0.275	0.413	0.962	0.305	0.459	2.388	0.756	1.138
		005	Dosing-Terminal Euthanasia	294.1	0.766	0.260	0.397	0.879	0.299	0.456	2.197	0.747	1.140
		006	Dosing-Terminal Euthanasia	304.8	0.589	0.193	0.318	0.915	0.300	0.494	2.216	0.727	1.197
		007	Dosing-Terminal Euthanasia	304.1	0.585	0.192	0.309	1.053	0.346	0.556	2.288	0.752	1.208
		008	Dosing-Terminal Euthanasia	286.7	0.756	0.264	0.430	0.890	0.310	0.506	1.900	0.663	1.080
		009	Dosing-Terminal Euthanasia	276.4	0.724	0.262	0.372	0.906	0.328	0.466	2.042	0.739	1.049
		010	Dosing-Terminal Euthanasia	287.8	0.591	0.205	0.316	0.776	0.270	0.415	2.030	0.705	1.087
		011	Recovery-Recovery Euthanasia 1	329.10	0.884	0.269	0.407	1.011	0.307	0.465	2.406	0.731	1.107
		012	Recovery-Recovery Euthanasia 1	362.80	1.159	0.319	0.557	1.124	0.310	0.540	2.724	0.751	1.310
		013	Recovery-Recovery Euthanasia 1	304.00	1.450	0.477	0.691	0.790	0.260	0.377	2.060	0.678	0.982
		014	Recovery-Recovery Euthanasia 1	283.80	0.874	0.308	0.426	0.898	0.316	0.438	2.025	0.714	0.987
		015	Recovery-Recovery Euthanasia 1	379.20	1.299	0.343	0.609	1.311	0.346	0.615	2.814	0.742	1.319

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Gland, Prostate			Heart			Kidney		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	016	Dosing-Terminal Euthanasia	257.4	0.697	0.271	0.395	0.964	0.375	0.546	1.965	0.763	1.113
		017	Dosing-Terminal Euthanasia	284.4	0.479	0.168	0.246	0.934	0.328	0.479	2.181	0.767	1.119
		018	Dosing-Terminal Euthanasia	267.3	0.520	0.195	0.283	0.801	0.300	0.436	2.195	0.821	1.194
		019	Dosing-Terminal Euthanasia	289.1	0.772	0.267	0.436	1.180	0.408	0.666	2.352	0.814	1.327
		020	Dosing-Terminal Euthanasia	250.2	0.658	0.263	0.328	0.880	0.352	0.439	2.020	0.807	1.007
		021	Dosing-Terminal Euthanasia	269.6	0.636	0.236	0.308	0.968	0.359	0.469	2.323	0.862	1.127
		022	Dosing-Terminal Euthanasia	268.5	0.683	0.254	0.391	0.838	0.312	0.480	2.064	0.769	1.182
		023	Dosing-Terminal Euthanasia	274.5	0.716	0.261	0.391	0.894	0.326	0.489	2.163	0.788	1.182
		024	Dosing-Terminal Euthanasia	248.5	0.950	0.382	0.467	0.785	0.316	0.386	2.182	0.878	1.073
		025	Dosing-Terminal Euthanasia	302.2	1.213	0.401	0.562	0.998	0.330	0.463	2.752	0.911	1.276
		026	Recovery-Recovery Euthanasia 1	351.50	0.869	0.247	0.479	1.038	0.295	0.572	2.282	0.649	1.257
		027	Recovery-Recovery Euthanasia 1	340.00	1.029	0.303	0.566	1.043	0.307	0.574	2.270	0.668	1.249
		028	Recovery-Recovery Euthanasia 1	335.40	1.306	0.389	0.628	1.152	0.343	0.554	2.440	0.727	1.173
		029	Recovery-Recovery Euthanasia 1	350.40	0.884	0.252	0.429	1.171	0.334	0.568	2.364	0.675	1.146
		030	Recovery-Recovery Euthanasia 1	380.20	1.008	0.265	0.499	1.091	0.287	0.540	2.347	0.617	1.162

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Gland, Prostate			Heart			Kidney		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	031	Dosing-Terminal Euthanasia	244.7	0.709	0.290	0.392	0.807	0.330	0.446	1.808	0.739	1.000
		032	Dosing-Terminal Euthanasia	254.6	0.580	0.228	0.306	0.805	0.316	0.424	1.998	0.785	1.053
		033	Dosing-Terminal Euthanasia	301.1	0.781	0.259	0.402	0.984	0.327	0.506	2.209	0.734	1.136
		034	Dosing-Terminal Euthanasia	250.4	0.500	0.200	0.271	0.837	0.334	0.454	1.720	0.687	0.934
		035	Dosing-Terminal Euthanasia	235.8	0.724	0.307	0.377	0.767	0.325	0.399	1.828	0.775	0.951
		036	Dosing-Terminal Euthanasia	278.8	0.767	0.275	0.404	0.898	0.322	0.473	2.325	0.834	1.225
		037	Dosing-Terminal Euthanasia	273.2	0.651	0.238	0.350	1.127	0.413	0.606	2.067	0.757	1.111
		038	Dosing-Terminal Euthanasia	258.7	0.545	0.211	0.279	0.853	0.330	0.437	2.221	0.859	1.138
		039	Dosing-Terminal Euthanasia	262.2	0.836	0.319	0.429	0.853	0.325	0.438	1.981	0.756	1.017
		040	Dosing-Terminal Euthanasia	266.4	0.662	0.248	0.329	0.864	0.324	0.430	2.095	0.786	1.042
		041	Recovery-Recovery Euthanasia 1	339.00	0.909	0.268	0.473	1.091	0.322	0.568	2.470	0.729	1.285
		042	Recovery-Recovery Euthanasia 1	325.50	0.823	0.253	0.419	0.958	0.294	0.488	1.963	0.603	1.001
		043	Recovery-Recovery Euthanasia 1	344.20	1.169	0.340	0.603	1.085	0.315	0.560	2.293	0.666	1.183
		044	Recovery-Recovery Euthanasia 1	347.50	1.088	0.313	0.561	1.088	0.313	0.561	2.331	0.671	1.203
		045	Recovery-Recovery Euthanasia 1	315.60	1.234	0.391	0.635	1.104	0.350	0.568	2.359	0.747	1.214

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Liver			Spleen			Testis		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	001	Dosing-Terminal Euthanasia	269.7	8.285	3.072	4.517	0.503	0.187	0.274	3.436	1.274	1.874
		002	Dosing-Terminal Euthanasia	321.3	9.334	2.905	4.834	0.669	0.208	0.346	3.283	1.022	1.700
		003	Dosing-Terminal Euthanasia	300.0	8.844	2.948	4.533	0.598	0.199	0.307	3.689	1.230	1.891
		004	Dosing-Terminal Euthanasia	315.7	8.552	2.709	4.076	0.689	0.218	0.328	3.599	1.140	1.715
		005	Dosing-Terminal Euthanasia	294.1	8.495	2.888	4.406	0.604	0.205	0.313	3.500	1.190	1.815
		006	Dosing-Terminal Euthanasia	304.8	8.099	2.657	4.375	0.537	0.176	0.290	2.828	0.928	1.528
		007	Dosing-Terminal Euthanasia	304.1	8.327	2.738	4.397	0.635	0.209	0.335	2.729	0.897	1.441
		008	Dosing-Terminal Euthanasia	286.7	7.583	2.645	4.309	0.607	0.212	0.345	3.189	1.112	1.812
		009	Dosing-Terminal Euthanasia	276.4	7.937	2.872	4.079	0.590	0.213	0.303	3.296	1.192	1.694
		010	Dosing-Terminal Euthanasia	287.8	7.762	2.697	4.155	0.519	0.180	0.278	3.178	1.104	1.701
		011	Recovery-Recovery Euthanasia 1	329.10	8.169	2.482	3.759	0.602	0.183	0.277	3.522	1.070	1.621
		012	Recovery-Recovery Euthanasia 1	362.80	9.132	2.517	4.390	0.647	0.178	0.311	3.802	1.048	1.828
		013	Recovery-Recovery Euthanasia 1	304.00	7.466	2.456	3.559	0.542	0.178	0.258	3.191	1.050	1.521
		014	Recovery-Recovery Euthanasia 1	283.80	7.437	2.621	3.624	0.655	0.231	0.319	3.421	1.205	1.667
		015	Recovery-Recovery Euthanasia 1	379.20	10.744	2.833	5.037	0.597	0.157	0.280	3.800	1.002	1.782

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Liver			Spleen			Testis		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	016	Dosing-Terminal Euthanasia	257.4	7.531	2.926	4.267	0.790	0.307	0.448	3.506	1.362	1.986
		017	Dosing-Terminal Euthanasia	284.4	7.900	2.778	4.053	0.782	0.275	0.401	3.547	1.247	1.820
		018	Dosing-Terminal Euthanasia	267.3	8.757	3.276	4.762	0.775	0.290	0.421	3.004	1.124	1.633
		019	Dosing-Terminal Euthanasia	289.1	8.317	2.877	4.694	0.626	0.217	0.353	3.508	1.213	1.980
		020	Dosing-Terminal Euthanasia	250.2	7.481	2.990	3.729	0.747	0.299	0.372	3.406	1.361	1.698
		021	Dosing-Terminal Euthanasia	269.6	7.714	2.861	3.741	0.933	0.346	0.452	3.582	1.329	1.737
		022	Dosing-Terminal Euthanasia	268.5	7.203	2.683	4.125	0.697	0.260	0.399	3.037	1.131	1.739
		023	Dosing-Terminal Euthanasia	274.5	7.457	2.717	4.075	0.767	0.279	0.419	3.480	1.268	1.902
		024	Dosing-Terminal Euthanasia	248.5	7.389	2.973	3.635	0.646	0.260	0.318	3.480	1.400	1.712
		025	Dosing-Terminal Euthanasia	302.2	8.131	2.691	3.770	0.937	0.310	0.434	4.133	1.368	1.916
		026	Recovery-Recovery Euthanasia 1	351.50	9.462	2.692	5.213	0.674	0.192	0.371	3.169	0.902	1.746
		027	Recovery-Recovery Euthanasia 1	340.00	8.740	2.571	4.807	0.710	0.209	0.391	2.955	0.869	1.625
		028	Recovery-Recovery Euthanasia 1	335.40	8.136	2.426	3.912	0.703	0.210	0.338	3.595	1.072	1.728
		029	Recovery-Recovery Euthanasia 1	350.40	9.145	2.610	4.435	0.727	0.207	0.353	3.828	1.092	1.856
		030	Recovery-Recovery Euthanasia 1	380.20	9.353	2.460	4.630	0.801	0.211	0.397	3.744	0.985	1.853

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Liver			Spleen			Testis		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	031	Dosing-Terminal Euthanasia	244.7	7.476	3.055	4.135	0.850	0.347	0.470	3.361	1.374	1.859
		032	Dosing-Terminal Euthanasia	254.6	7.377	2.897	3.889	0.775	0.304	0.409	3.475	1.365	1.832
		033	Dosing-Terminal Euthanasia	301.1	8.757	2.908	4.502	0.838	0.278	0.431	2.980	0.990	1.532
		034	Dosing-Terminal Euthanasia	250.4	6.773	2.705	3.677	0.680	0.272	0.369	2.942	1.175	1.597
		035	Dosing-Terminal Euthanasia	235.8	7.037	2.984	3.661	0.799	0.339	0.416	3.422	1.451	1.780
		036	Dosing-Terminal Euthanasia	278.8	7.802	2.798	4.111	0.736	0.264	0.388	3.126	1.121	1.647
		037	Dosing-Terminal Euthanasia	273.2	8.310	3.042	4.465	0.976	0.357	0.524	3.090	1.131	1.660
		038	Dosing-Terminal Euthanasia	258.7	7.656	2.959	3.924	0.883	0.341	0.453	3.263	1.261	1.672
		039	Dosing-Terminal Euthanasia	262.2	7.155	2.729	3.675	0.730	0.278	0.375	3.445	1.314	1.769
		040	Dosing-Terminal Euthanasia	266.4	7.529	2.826	3.744	0.717	0.269	0.357	3.612	1.356	1.796
		041	Recovery-Recovery Euthanasia 1	339.00	8.953	2.641	4.658	0.478	0.141	0.249	3.415	1.007	1.777
		042	Recovery-Recovery Euthanasia 1	325.50	8.199	2.519	4.179	0.671	0.206	0.342	4.027	1.237	2.052
		043	Recovery-Recovery Euthanasia 1	344.20	8.854	2.572	4.566	0.624	0.181	0.322	3.508	1.019	1.809
		044	Recovery-Recovery Euthanasia 1	347.50	9.468	2.725	4.885	0.782	0.225	0.404	3.825	1.101	1.974
		045	Recovery-Recovery Euthanasia 1	315.60	8.320	2.636	4.282	0.747	0.237	0.384	3.694	1.170	1.901

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

					Thymus		
Group Number	**Dose**	**Animal Number**	**Phase-Planned Sacrifice Group**	**BW (g)**	**ABS**	**OW:BW**	**OW:BRN**
1	0 µg/day	001	Dosing-Terminal Euthanasia	269.7	0.581	0.215	0.317
		002	Dosing-Terminal Euthanasia	321.3	0.611	0.190	0.316
		003	Dosing-Terminal Euthanasia	300.0	0.643	0.214	0.330
		004	Dosing-Terminal Euthanasia	315.7	0.681	0.216	0.325
		005	Dosing-Terminal Euthanasia	294.1	0.522	0.177	0.271
		006	Dosing-Terminal Euthanasia	304.8	0.556	0.182	0.300
		007	Dosing-Terminal Euthanasia	304.1	0.657	0.216	0.347
		008	Dosing-Terminal Euthanasia	286.7	0.484	0.169	0.275
		009	Dosing-Terminal Euthanasia	276.4	0.653	0.236	0.336
		010	Dosing-Terminal Euthanasia	287.8	0.526	0.183	0.282
		011	Recovery-Recovery Euthanasia 1	329.10	0.397	0.121	0.183
		012	Recovery-Recovery Euthanasia 1	362.80	0.407	0.112	0.196
		013	Recovery-Recovery Euthanasia 1	304.00	0.519	0.171	0.247
		014	Recovery-Recovery Euthanasia 1	283.80	0.583	0.205	0.284
		015	Recovery-Recovery Euthanasia 1	379.20	0.563	0.148	0.264

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

					Thymus		
Group Number	**Dose**	**Animal Number**	**Phase-Planned Sacrifice Group**	**BW (g)**	**ABS**	**OW:BW**	**OW:BRN**
2	30 µg/day	016	Dosing-Terminal Euthanasia	257.4	0.351	0.136	0.199
		017	Dosing-Terminal Euthanasia	284.4	0.650	0.229	0.334
		018	Dosing-Terminal Euthanasia	267.3	0.466	0.174	0.253
		019	Dosing-Terminal Euthanasia	289.1	0.412	0.143	0.233
		020	Dosing-Terminal Euthanasia	250.2	0.409	0.163	0.204
		021	Dosing-Terminal Euthanasia	269.6	0.479	0.178	0.232
		022	Dosing-Terminal Euthanasia	268.5	0.562	0.209	0.322
		023	Dosing-Terminal Euthanasia	274.5	0.469	0.171	0.256
		024	Dosing-Terminal Euthanasia	248.5	0.353	0.142	0.174
		025	Dosing-Terminal Euthanasia	302.2	0.522	0.173	0.242
		026	Recovery-Recovery Euthanasia 1	351.50	0.572	0.163	0.315
		027	Recovery-Recovery Euthanasia 1	340.00	0.492	0.145	0.271
		028	Recovery-Recovery Euthanasia 1	335.40	0.530	0.158	0.255
		029	Recovery-Recovery Euthanasia 1	350.40	0.525	0.150	0.255
		030	Recovery-Recovery Euthanasia 1	380.20	0.649	0.171	0.321

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Male

					Thymus		
Group Number	**Dose**	**Animal Number**	**Phase-Planned Sacrifice Group**	**BW (g)**	**ABS**	**OW:BW**	**OW:BRN**
3	30 µg /day	031	Dosing-Terminal Euthanasia	244.7	0.306	0.125	0.169
		032	Dosing-Terminal Euthanasia	254.6	0.550	0.216	0.290
		033	Dosing-Terminal Euthanasia	301.1	0.573	0.190	0.295
		034	Dosing-Terminal Euthanasia	250.4	0.332	0.133	0.180
		035	Dosing-Terminal Euthanasia	235.8	0.343	0.145	0.178
		036	Dosing-Terminal Euthanasia	278.8	0.460	0.165	0.242
		037	Dosing-Terminal Euthanasia	273.2	0.454	0.166	0.244
		038	Dosing-Terminal Euthanasia	258.7	0.359	0.139	0.184
		039	Dosing-Terminal Euthanasia	262.2	0.396	0.151	0.203
		040	Dosing-Terminal Euthanasia	266.4	0.427	0.160	0.212
		041	Recovery-Recovery Euthanasia 1	339.00	0.348	0.103	0.181
		042	Recovery-Recovery Euthanasia 1	325.50	0.525	0.161	0.268
		043	Recovery-Recovery Euthanasia 1	344.20	0.366	0.106	0.189
		044	Recovery-Recovery Euthanasia 1	347.50	0.416	0.120	0.215
		045	Recovery-Recovery Euthanasia 1	315.60	0.480	0.152	0.247

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Gland, Adrenal			Heart		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	046	Dosing-Terminal Euthanasia	185.6	1.734	0.934	1.000	0.067	0.036	0.039	0.670	0.361	0.386
		047	Dosing-Terminal Euthanasia	213.7	1.948	0.912	1.000	0.086	0.040	0.044	0.807	0.378	0.414
		048	Dosing-Terminal Euthanasia	183.1	1.928	1.053	1.000	0.075	0.041	0.039	0.757	0.413	0.393
		049	Dosing-Terminal Euthanasia	192.4	1.836	0.954	1.000	0.067	0.035	0.036	0.659	0.343	0.359
		050	Dosing-Terminal Euthanasia	206.6	1.913	0.926	1.000	0.107	0.052	0.056	0.640	0.310	0.335
		051	Dosing-Terminal Euthanasia	207.2	1.837	0.887	1.000	0.100	0.048	0.054	0.865	0.417	0.471
		052	Dosing-Terminal Euthanasia	201.7	1.905	0.944	1.000	0.094	0.047	0.049	0.738	0.366	0.387
		053	Dosing-Terminal Euthanasia	202.0	1.907	0.944	1.000	0.080	0.040	0.042	0.833	0.412	0.437
		054	Dosing-Terminal Euthanasia	207.8	1.797	0.865	1.000	0.114	0.055	0.063	0.803	0.386	0.447
		055	Dosing-Terminal Euthanasia	187.2	1.805	0.964	1.000	0.092	0.049	0.051	0.678	0.362	0.376
		056	Recovery-Recovery Euthanasia 1	230.00	1.854	0.806	1.000	0.093	0.040	0.050	0.759	0.330	0.409
		057	Recovery-Recovery Euthanasia 1	202.70	1.804	0.890	1.000	0.077	0.038	0.043	0.689	0.340	0.382
		058	Recovery-Recovery Euthanasia 1	203.30	1.668	0.820	1.000	0.084	0.041	0.050	0.702	0.345	0.421
		059	Recovery-Recovery Euthanasia 1	200.80	1.938	0.965	1.000	0.100	0.050	0.052	0.691	0.344	0.357
		060	Recovery-Recovery Euthanasia 1	231.40	1.904	0.823	1.000	0.079	0.034	0.041	0.815	0.352	0.428

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Gland, Adrenal			Heart		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	061	Dosing-Terminal Euthanasia	201.9	1.753	0.868	1.000	0.091	0.045	0.052	0.842	0.417	0.480
		062	Dosing-Terminal Euthanasia	188.0	1.846	0.982	1.000	0.076	0.040	0.041	0.733	0.390	0.397
		063	Dosing-Terminal Euthanasia	180.5	1.678	0.930	1.000	0.082	0.045	0.049	0.875	0.485	0.521
		064	Dosing-Terminal Euthanasia	197.0	1.834	0.931	1.000	0.096	0.049	0.052	0.699	0.355	0.381
		065	Dosing-Terminal Euthanasia	196.8	1.808	0.919	1.000	0.073	0.037	0.040	0.733	0.372	0.405
		066	Dosing-Terminal Euthanasia	194.9	1.844	0.946	1.000	0.076	0.039	0.041	0.669	0.343	0.363
		067	Dosing-Terminal Euthanasia	198.0	1.845	0.932	1.000	0.113	0.057	0.061	0.775	0.391	0.420
		068	Dosing-Terminal Euthanasia	175.3	1.713	0.977	1.000	0.069	0.039	0.040	0.607	0.346	0.354
		069	Dosing-Terminal Euthanasia	211.6	1.762	0.833	1.000	0.108	0.051	0.061	0.861	0.407	0.489
		070	Dosing-Terminal Euthanasia	201.6	1.785	0.885	1.000	0.102	0.051	0.057	0.779	0.386	0.436
		071	Recovery-Recovery Euthanasia 1	216.00	2.093	0.969	1.000	0.088	0.041	0.042	0.778	0.360	0.372
		072	Recovery-Recovery Euthanasia 1	216.00	1.759	0.814	1.000	0.070	0.032	0.040	0.939	0.435	0.534
		073	Recovery-Recovery Euthanasia 1	205.20	1.900	0.926	1.000	0.087	0.042	0.046	0.690	0.336	0.363
		074	Recovery-Recovery Euthanasia 1	209.20	1.850	0.884	1.000	0.104	0.050	0.056	0.890	0.425	0.481
		075	Recovery-Recovery Euthanasia 1	220.40	1.764	0.800	1.000	0.103	0.047	0.058	1.032	0.468	0.585

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Brain			Gland, Adrenal			Heart		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	076	Dosing-Terminal Euthanasia	192.2	1.758	0.915	1.000	0.024e	0.012e	0.014e	0.686	0.357	0.390
		077	Dosing-Terminal Euthanasia	190.0	1.757	0.925	1.000	0.074	0.039	0.042	0.748	0.394	0.426
		078	Dosing-Terminal Euthanasia	201.5	1.899	0.942	1.000	0.094	0.047	0.049	0.843	0.418	0.444
		079	Dosing-Terminal Euthanasia	200.8	1.806	0.899	1.000	0.095	0.047	0.053	0.720	0.359	0.399
		080	Dosing-Terminal Euthanasia	199.4	1.896	0.951	1.000	0.113	0.057	0.060	0.707	0.355	0.373
		081	Dosing-Terminal Euthanasia	185.4	1.868	1.008	1.000	0.069	0.037	0.037	0.615	0.332	0.329
		082	Dosing-Terminal Euthanasia	184.3	1.842	0.999	1.000	0.072	0.039	0.039	0.640	0.347	0.347
		083	Dosing-Terminal Euthanasia	192.1	1.822	0.948	1.000	0.124	0.065	0.068	0.881	0.459	0.484
		084	Dosing-Terminal Euthanasia	191.9	2.001	1.043	1.000	0.097	0.051	0.048	0.678	0.353	0.339
		085	Dosing-Terminal Euthanasia	180.6	1.758	0.973	1.000	0.078	0.043	0.044	0.655	0.363	0.373
		086	Recovery-Recovery Euthanasia 1	210.30	1.785	0.849	1.000	0.099	0.047	0.055	0.719	0.342	0.403
		087	Recovery-Recovery Euthanasia 1	237.80	1.819	0.765	1.000	0.092	0.039	0.051	0.930	0.391	0.511
		088	Recovery-Recovery Euthanasia 1	182.40	1.918	1.052	1.000	0.089	0.049	0.046	0.760	0.417	0.396
		089	Recovery-Recovery Euthanasia 1	212.50	1.862	0.876	1.000	0.079	0.037	0.042	0.776	0.365	0.417
		090	Recovery-Recovery Euthanasia 1	208.80	1.841	0.882	1.000	0.091	0.044	0.049	0.920	0.441	0.500

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Kidney			Liver			Ovary		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	046	Dosing-Terminal Euthanasia	185.6	1.528	0.823	0.881	5.132	2.765	2.960	0.105	0.057	0.061
		047	Dosing-Terminal Euthanasia	213.7	1.491	0.698	0.765	6.077	2.844	3.120	0.126	0.059	0.065
		048	Dosing-Terminal Euthanasia	183.1	1.521	0.831	0.789	4.935	2.695	2.560	0.124	0.068	0.064
		049	Dosing-Terminal Euthanasia	192.4	1.416	0.736	0.771	5.284	2.746	2.878	0.103	0.054	0.056
		050	Dosing-Terminal Euthanasia	206.6	1.638	0.793	0.856	5.809	2.812	3.037	0.129	0.062	0.067
		051	Dosing-Terminal Euthanasia	207.2	1.545	0.746	0.841	5.489	2.649	2.988	0.085	0.041	0.046
		052	Dosing-Terminal Euthanasia	201.7	1.563	0.775	0.820	5.626	2.789	2.953	0.118	0.059	0.062
		053	Dosing-Terminal Euthanasia	202.0	1.514	0.750	0.794	5.556	2.750	2.913	0.130	0.064	0.068
		054	Dosing-Terminal Euthanasia	207.8	1.653	0.795	0.920	5.314	2.557	2.957	0.137	0.066	0.076
		055	Dosing-Terminal Euthanasia	187.2	1.404	0.750	0.778	5.349	2.857	2.963	0.110	0.059	0.061
		056	Recovery-Recovery Euthanasia 1	230.00	1.694	0.737	0.914	6.030	2.622	3.252	0.149	0.065	0.080
		057	Recovery-Recovery Euthanasia 1	202.70	1.340	0.661	0.743	5.280	2.605	2.927	0.090	0.044	0.050
		058	Recovery-Recovery Euthanasia 1	203.30	1.392	0.685	0.835	5.299	2.606	3.177	0.097	0.048	0.058
		059	Recovery-Recovery Euthanasia 1	200.80	1.560	0.777	0.805	5.176	2.578	2.671	0.114	0.057	0.059
		060	Recovery-Recovery Euthanasia 1	231.40	1.660	0.717	0.872	6.028	2.605	3.166	0.171	0.074	0.090

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Kidney			Liver			Ovary		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	061	Dosing-Terminal Euthanasia	201.9	1.579	0.782	0.901	6.265	3.103	3.574	0.121	0.060	0.069
		062	Dosing-Terminal Euthanasia	188.0	1.595	0.848	0.864	5.849	3.111	3.168	0.071	0.038	0.038
		063	Dosing-Terminal Euthanasia	180.5	1.550	0.859	0.924	4.987	2.763	2.972	0.087	0.048	0.052
		064	Dosing-Terminal Euthanasia	197.0	1.528	0.776	0.833	5.364	2.723	2.925	0.106	0.054	0.058
		065	Dosing-Terminal Euthanasia	196.8	1.621	0.824	0.897	6.048	3.073	3.345	0.120	0.061	0.066
		066	Dosing-Terminal Euthanasia	194.9	1.662	0.853	0.901	5.055	2.594	2.741	0.115	0.059	0.062
		067	Dosing-Terminal Euthanasia	198.0	1.720	0.869	0.932	5.548	2.802	3.007	0.124	0.063	0.067
		068	Dosing-Terminal Euthanasia	175.3	1.614	0.921	0.942	4.941	2.819	2.884	0.121	0.069	0.071
		069	Dosing-Terminal Euthanasia	211.6	1.766	0.835	1.002	6.512	3.078	3.696	0.095	0.045	0.054
		070	Dosing-Terminal Euthanasia	201.6	1.708	0.847	0.957	5.921	2.937	3.317	0.093	0.046	0.052
		071	Recovery-Recovery Euthanasia 1	216.00	1.620	0.750	0.774	5.605	2.595	2.678	0.125	0.058	0.060
		072	Recovery-Recovery Euthanasia 1	216.00	1.591	0.737	0.904	6.056	2.804	3.443	0.078	0.036	0.044
		073	Recovery-Recovery Euthanasia 1	205.20	1.482	0.722	0.780	5.460	2.661	2.874	0.170	0.083	0.089
		074	Recovery-Recovery Euthanasia 1	209.20	1.723	0.824	0.931	5.655	2.703	3.057	0.166	0.079	0.090
		075	Recovery-Recovery Euthanasia 1	220.40	1.828	0.829	1.036	6.258	2.839	3.548	0.113	0.051	0.064

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Kidney			Liver			Ovary		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	076	Dosing-Terminal Euthanasia	192.2	1.596	0.830	0.908	5.669	2.950	3.225	0.015e	0.008e	0.009e
		077	Dosing-Terminal Euthanasia	190.0	1.529	0.805	0.870	5.637	2.967	3.208	0.092	0.048	0.052
		078	Dosing-Terminal Euthanasia	201.5	1.750	0.868	0.922	6.241	3.097	3.286	0.116	0.058	0.061
		079	Dosing-Terminal Euthanasia	200.8	1.794	0.893	0.993	6.541	3.257	3.622	0.127	0.063	0.070
		080	Dosing-Terminal Euthanasia	199.4	1.684	0.845	0.888	6.451	3.235	3.402	0.139	0.070	0.073
		081	Dosing-Terminal Euthanasia	185.4	1.669	0.900	0.893	5.539	2.988	2.965	0.116	0.063	0.062
		082	Dosing-Terminal Euthanasia	184.3	1.402	0.761	0.761	5.071	2.751	2.753	0.096	0.052	0.052
		083	Dosing-Terminal Euthanasia	192.1	1.756	0.914	0.964	5.806	3.022	3.187	0.119	0.062	0.065
		084	Dosing-Terminal Euthanasia	191.9	1.590	0.829	0.795	5.941	3.096	2.969	0.110	0.057	0.055
		085	Dosing-Terminal Euthanasia	180.6	1.394	0.772	0.793	5.208	2.884	2.962	0.087	0.048	0.049
		086	Recovery-Recovery Euthanasia 1	210.30	1.615	0.768	0.905	5.322	2.531	2.982	0.147	0.070	0.082
		087	Recovery-Recovery Euthanasia 1	237.80	1.823	0.767	1.002	6.706	2.820	3.687	0.169	0.071	0.093
		088	Recovery-Recovery Euthanasia 1	182.40	1.725	0.946	0.899	5.371	2.945	2.800	0.119	0.065	0.062
		089	Recovery-Recovery Euthanasia 1	212.50	1.737	0.817	0.933	5.787	2.723	3.108	0.112	0.053	0.060
		090	Recovery-Recovery Euthanasia 1	208.80	1.670	0.800	0.907	5.952	2.851	3.233	0.112	0.054	0.061

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Spleen			Thymus		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
1	0 µg/day	046	Dosing-Terminal Euthanasia	185.6	0.356	0.192	0.205	0.457	0.246	0.264
		047	Dosing-Terminal Euthanasia	213.7	0.501	0.234	0.257	0.426	0.199	0.219
		048	Dosing-Terminal Euthanasia	183.1	0.348	0.190	0.180	0.373	0.204	0.193
		049	Dosing-Terminal Euthanasia	192.4	0.516	0.268	0.281	0.538	0.280	0.293
		050	Dosing-Terminal Euthanasia	206.6	0.490	0.237	0.256	0.573	0.277	0.300
		051	Dosing-Terminal Euthanasia	207.2	0.416	0.201	0.226	0.413	0.199	0.225
		052	Dosing-Terminal Euthanasia	201.7	0.532	0.264	0.279	0.527	0.261	0.277
		053	Dosing-Terminal Euthanasia	202.0	0.389	0.193	0.204	0.396	0.196	0.208
		054	Dosing-Terminal Euthanasia	207.8	0.425	0.205	0.237	0.493	0.237	0.274
		055	Dosing-Terminal Euthanasia	187.2	0.409	0.218	0.227	0.392	0.209	0.217
		056	Recovery-Recovery Euthanasia 1	230.00	0.565	0.246	0.305	0.410	0.178	0.221
		057	Recovery-Recovery Euthanasia 1	202.70	0.413	0.204	0.229	0.392	0.193	0.217
		058	Recovery-Recovery Euthanasia 1	203.30	0.332	0.163	0.199	0.496	0.244	0.297
		059	Recovery-Recovery Euthanasia 1	200.80	0.380	0.189	0.196	0.336	0.167	0.173
		060	Recovery-Recovery Euthanasia 1	231.40	0.516	0.223	0.271	0.505	0.218	0.265

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Spleen			Thymus		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
2	30 µg/day	061	Dosing-Terminal Euthanasia	201.9	0.712	0.353	0.406	0.447	0.221	0.255
		062	Dosing-Terminal Euthanasia	188.0	0.694	0.369	0.376	0.380	0.202	0.206
		063	Dosing-Terminal Euthanasia	180.5	0.640	0.355	0.381	0.530	0.294	0.316
		064	Dosing-Terminal Euthanasia	197.0	0.884	0.449	0.482	0.372	0.189	0.203
		065	Dosing-Terminal Euthanasia	196.8	0.785	0.399	0.434	0.400	0.203	0.221
		066	Dosing-Terminal Euthanasia	194.9	0.552	0.283	0.299	0.495	0.254	0.268
		067	Dosing-Terminal Euthanasia	198.0	0.598	0.302	0.324	0.387	0.195	0.210
		068	Dosing-Terminal Euthanasia	175.3	0.552	0.315	0.322	0.121	0.069	0.071
		069	Dosing-Terminal Euthanasia	211.6	0.674	0.319	0.383	0.478	0.226	0.271
		070	Dosing-Terminal Euthanasia	201.6	0.705	0.350	0.395	0.357	0.177	0.200
		071	Recovery-Recovery Euthanasia 1	216.00	0.476	0.220	0.227	0.429	0.199	0.205
		072	Recovery-Recovery Euthanasia 1	216.00	0.411	0.190	0.234	0.463	0.214	0.263
		073	Recovery-Recovery Euthanasia 1	205.20	0.482	0.235	0.254	0.406	0.198	0.214
		074	Recovery-Recovery Euthanasia 1	209.20	0.504	0.241	0.272	0.460	0.220	0.249
		075	Recovery-Recovery Euthanasia 1	220.40	0.500	0.227	0.283	0.431	0.196	0.244

Appendix 10

Organ Weights (g) and Ratios

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Female

Group Number	Dose	Animal Number	Phase-Planned Sacrifice Group	BW (g)	Spleen			Thymus		
					ABS	OW:BW	OW:BRN	ABS	OW:BW	OW:BRN
3	30 µg /day	076	Dosing-Terminal Euthanasia	192.2	0.601	0.313	0.342	0.339	0.176	0.193
		077	Dosing-Terminal Euthanasia	190.0	0.583	0.307	0.332	0.399	0.210	0.227
		078	Dosing-Terminal Euthanasia	201.5	0.610	0.303	0.321	0.374	0.186	0.197
		079	Dosing-Terminal Euthanasia	200.8	0.712	0.355	0.394	0.410	0.204	0.227
		080	Dosing-Terminal Euthanasia	199.4	0.635	0.318	0.335	0.518	0.260	0.273
		081	Dosing-Terminal Euthanasia	185.4	0.543	0.293	0.291	0.419	0.226	0.224
		082	Dosing-Terminal Euthanasia	184.3	0.677	0.367	0.368	0.363	0.197	0.197
		083	Dosing-Terminal Euthanasia	192.1	0.684	0.356	0.375	0.402	0.209	0.221
		084	Dosing-Terminal Euthanasia	191.9	0.587	0.306	0.293	0.295	0.154	0.147
		085	Dosing-Terminal Euthanasia	180.6	0.567	0.314	0.323	0.387	0.214	0.220
		086	Recovery-Recovery Euthanasia 1	210.30	0.378	0.180	0.212	0.413	0.196	0.231
		087	Recovery-Recovery Euthanasia 1	237.80	0.532	0.224	0.292	0.425	0.179	0.234
		088	Recovery-Recovery Euthanasia 1	182.40	0.347	0.190	0.181	0.317	0.174	0.165
		089	Recovery-Recovery Euthanasia 1	212.50	0.461	0.217	0.248	0.418	0.197	0.224
		090	Recovery-Recovery Euthanasia 1	208.80	0.518	0.248	0.281	0.388	0.186	0.211

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Footnotes

NOS = Not otherwise specified.

Note: All tissues are considered as macroscopically unremarkable unless noted in the individual animal listings.

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
001	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Site, Injection	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Testis	Thymus	Tongue	Trachea	Ureter
Urinary Bladder				

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
002	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gland, Thyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Lymph Node, Inguinal	No macroscopic observations on tissue		Missing

The following required protocol tissues were not examined microscopically:

Lymph Node, Inguinal

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Pancreas	Site, Injection	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
003	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Kidney	No macroscopic observations on tissue		Dilatation, Pelvis, Unilateral, Minimal
Lung	Abnormal color, Dark, Focal, 0.3-0.5 cm, Left lobe	Not Correlated	Tissue is unremarkable
Lymph Node, Inguinal	Abnormal size, Enlarged, Right	Not Correlated	Tissue is unremarkable
Nerve, Optic	No macroscopic observations on tissue		Tissue Comment: examined along with the eye sections
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
003	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lymph Node, Draining	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
004	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
005	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
006	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Site, Injection	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Spleen	Stomach	Testis	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
007	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Pancreas	Site, Injection	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
008	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
009	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Epididymis
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary
Gland, Seminal Vesicle	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Site, Injection	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Testis	Thymus	Tongue	Trachea	Ureter
Urinary Bladder				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
010	M	1	0 µg/day	17	Dosing	Terminal Euthanasia

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain
Epididymis	Esophagus	Eye	Gland, Adrenal
Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Liver
Lung	Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Site, Injection	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder		

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
011	M	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Missing
Adipose Tissue	Abnormal color, Dark, Focal /Comments: Abdominal	Correlated	Inflammation, Mild
	Abnormal consistency, Firm, Focal	Correlated	Fibrosis, Minimal
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Mild

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Site, Injection

Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
012	M	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Draining Site, Injection
Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
013	M	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Draining Site, Injection
Spleen

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
014	M	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum	Joint	Liver	Lymph Node, Draining
Lymph Node, Inguinal	Site, Injection	Spleen	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
015	M	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum	Joint	Liver	Lymph Node, Draining
Lymph Node, Inguinal	Site, Injection	Spleen	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
016	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
016	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Testis	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
017	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver			Vacuolation, Hepatocyte; Periportal, Minimal
	Abnormal surface, Raised, Focal, < 0.3 cm	Not Correlated	
Lung			Tissue is unremarkable
	Abnormal color, Pale, Focal, Right caudal lobe	Not Correlated	
Lymph Node, Draining	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Germinal center, Mild
	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Plasma cell, Mild
Site, Injection	Abnormal color, Dark, Focal, 0.3-0.5 cm	Correlated	Edema, Mild
	Abnormal color, Dark, Focal, 0.3-0.5 cm	Correlated	Inflammation, Moderate
	Abnormal consistency, Firm, Focal	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
			Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
017	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Testis	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
018	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Pars anterior (distalis), Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Moderate
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
018	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Inguinal	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach
Testis	Thymus	Tongue	Trachea	Ureter
Urinary Bladder				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
019	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Kidney	No macroscopic observations on tissue		Tubular basophilia, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
019	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
020	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	Abnormal consistency, Firm, Focal, Site, Injection, 9 Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated Correlated	Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
020	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
021	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Mammary	No macroscopic observations on tissue		Missing
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Missing
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Moderate Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		Both Missing

The following required protocol tissues were not examined microscopically:

Gland, Mammary Lymph Node, Draining Ureter

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
021	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Parathyroid
Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Testis	Thymus
Tongue	Trachea	Urinary Bladder		

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
022	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Site, Injection	Abnormal color, Pale, Diffuse Abnormal color, Pale, Diffuse	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
022	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
023	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
023	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Draining
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
024	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
024	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
025	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Moderate
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
025	M	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
026	M	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node,	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Minimal
Draining	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Minimal
	Abnormal size, Enlarged	Correlated	Infiltration, Macrophage, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
027	M	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
028	M	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
029	M	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
030	M	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
031	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Pars anterior (distalis), Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Moderate Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
031	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
032	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
032	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
033	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
033	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
034	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
034	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
035	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Pars anterior (distalis), Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Site, Injection	Abnormal consistency, Firm, Focal Abnormal consistency, Firm, Focal	Correlated Correlated	Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal
Stomach	No macroscopic observations on tissue		Erosion, Nonglandular mucosa, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
035	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Testis	Thymus	Tongue
Trachea	Ureter	Urinary Bladder		

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
036	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Prostate	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
036	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Seminal Vesicle	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
037	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Unilateral, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
037	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Large Intestine, Cecum
Large Intestine, Colon	Lung	Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach
Testis	Thymus	Tongue	Trachea	Ureter
Urinary Bladder				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
038	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
038	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
039	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	Abnormal consistency, Firm, Focal, Site, Injection, 9 Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
039	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Testis
Thymus	Tongue	Trachea	Ureter	Urinary Bladder

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
040	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Mild
Site, Injection	Abnormal color, Pale, Diffuse Abnormal color, Pale, Diffuse	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
040	M	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Epididymis	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Prostate	Gland, Salivary	Gland, Seminal Vesicle
Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Stomach	Testis	Thymus	Tongue	Trachea
Ureter	Urinary Bladder			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
041	M	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
042	M	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
043	M	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
044	M	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Infiltration, Macrophage, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
045	M	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
046	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Adrenal	No macroscopic observations on tissue		Tissue Comment: medulla missing unilaterally
Site, Injection	Abnormal color, Dark, Focal, < 0.3 cm, Site, Injection, 9	Correlated	Inflammation, Minimal
Urinary Bladder	No macroscopic observations on tissue		Missing

The following required protocol tissues were not examined microscopically:

Urinary Bladder

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Thymus
Tongue	Trachea	Ureter	Uterus	Vagina

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
047	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
048	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Eye	No macroscopic observations on tissue		Rosettes retina, Minimal
Gland, Harderian	No macroscopic observations on tissue		Degeneration/Necrosis, Minimal Infiltration mononuclear cell, Minimal
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Joint	Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Site, Injection	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen
Stomach	Thymus	Tongue	Trachea	Ureter
Urinary Bladder	Uterus	Vagina		

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
049	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Harderian	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Minimal
Gland, Salivary	No macroscopic observations on tissue		Hypertrophy, Minimal
Gut-Associated Lymphoid Tissue	No macroscopic observations on tissue		Missing

The following required protocol tissues were not examined microscopically:

Gut-Associated
Lymphoid Tissue

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Thyroid	Heart	Joint	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Draining
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Site, Injection	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen
Stomach	Thymus	Tongue	Trachea	Ureter
Urinary Bladder	Uterus	Vagina		

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
050	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Adrenal	No macroscopic observations on tissue		Tissue Comment: Adrenal medulla missing unilaterally
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gland, Pituitary	No macroscopic observations on tissue		Tissue Comment: Pars distalis only
Gut-Associated Lymphoid Tissue	No macroscopic observations on tissue		Missing
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

Gut-Associated
Lymphoid Tissue

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
050	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Spleen	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
051	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Adrenal	No macroscopic observations on tissue		Tissue Comment: Adrenal medulla missing unilaterally
Gland, Harderian	No macroscopic observations on tissue		Degeneration/Necrosis, Minimal
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
051	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Large Intestine, Cecum	Large Intestine, Colon	Liver
Lung	Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct	Pancreas
Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord
Spleen	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
052	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Site, Injection	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
053	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Joint	No macroscopic observations on tissue		Physeal dysplasia, Focal, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Site, Injection	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
054	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Pituitary	No macroscopic observations on tissue		Tissue Comment: Pars distalis only

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital
Gland, Mammary	Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid
Gut-Associated Lymphoid Tissue	Heart	Joint	Kidney	Large Intestine, Cecum
Large Intestine, Colon	Liver	Lung	Lymph Node, Draining	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Site, Injection	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
055	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Gland, Harderian	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Lung	No macroscopic observations on tissue		Infiltration mixed cell, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
055	F	1	0 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone Marrow, Sternum	Bone, Sternum	Brain	Cervix
Esophagus	Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Liver	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Spleen	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
056	F	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum	Joint	Liver	Lymph Node, Draining
Lymph Node, Inguinal	Site, Injection	Spleen	

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
057	F	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Draining Site, Injection
Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
058	F	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum	Joint	Liver	Lymph Node, Draining
Lymph Node, Inguinal	Site, Injection	Spleen	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
059	F	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum	Joint	Liver	Lymph Node, Draining
Lymph Node, Inguinal	Site, Injection	Spleen	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
060	F	1	0 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Site, Injection Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
061	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Moderate
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal
Stomach	No macroscopic observations on tissue		Infiltration mononuclear cell, Serosa, Focal, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
061	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Lung	Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Ovary	Oviduct	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
062	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gland, Pituitary	No macroscopic observations on tissue		Tissue Comment: Missing, pars nervosa
Kidney	No macroscopic observations on tissue		Tubular basophilia, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Moderate
Oviduct	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Pancreas	No macroscopic observations on tissue		Atrophy, Acinar cell, Minimal Infiltration mononuclear cell, Interstitium, Focal, Minimal
Site, Injection	Abnormal consistency, Firm, Focal, Site, Injection, 9 Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
062	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
063	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Mammary	No macroscopic observations on tissue		Missing
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Moderate
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Skin	No macroscopic observations on tissue		Missing
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

Gland, Mammary Skin

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
063	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
064	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Pancreas	No macroscopic observations on tissue		Atrophy, Acinar cell, Minimal
Site, Injection	Abnormal consistency, Firm, Focal Abnormal consistency, Firm, Focal	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
064	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
065	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Pancreas	No macroscopic observations on tissue		Atrophy, Acinar cell, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
065	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
066	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal color, Pale, Diffuse	Correlated	Edema, Mild
	Abnormal color, Pale, Diffuse	Correlated	Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
066	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Draining	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
067	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lung	No macroscopic observations on tissue		Infiltration mixed cell, Focal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal
Site, Injection	Abnormal consistency, Firm, Focal Abnormal consistency, Firm, Focal	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
067	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
068	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
068	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
069	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Eye	No macroscopic observations on tissue		Mineralization, Cornea, Focal, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gut-Associated Lymphoid Tissue	No macroscopic observations on tissue		Mineralization, Germinal center, Focal, Minimal /Comments: associated with fibrosis and mixed inflammation
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Mild
Site, Injection	Abnormal color, Dark, Focal, 1-2 cm, Site, Injection, 9	Correlated	Edema, Mild
	Abnormal color, Dark, Focal, 1-2 cm, Site, Injection, 9	Correlated	Inflammation, Mild
	Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated	Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
069	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Inguinal
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
070	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Harderian	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Kidney	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Germinal center, Mild
	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
			Increased cellularity, Plasma cell, Minimal
Pancreas	No macroscopic observations on tissue		Atrophy, Acinar cell, Minimal
Site, Injection	Abnormal color, Pale, Diffuse	Correlated	Edema, Moderate
	Abnormal color, Pale, Diffuse	Correlated	Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
			Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
070	F	2	30 µg/day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Ureter	No macroscopic observations on tissue		Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric	Muscle, Skeletal
Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
071	F	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Adipose Tissue	Abnormal color, Dark, Multifocal /Comments: Neck, Ventral, Yellow	Correlated	Infiltration mononuclear cell, Mild /Comments: hemosiderophages

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
072	F	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
073	F	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
074	F	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Joint	No macroscopic observations on tissue		Physeal dysplasia, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Liver Lymph Node, Inguinal

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
075	F	2	30 µg/day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Infiltration, Macrophage, Mild
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal Spleen

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
076	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Eye	No macroscopic observations on tissue		Rosettes retina, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Mild
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
076	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Inguinal	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
077	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Harderian	No macroscopic observations on tissue		Degeneration/Necrosis, Minimal
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Plasma cell, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal consistency, Firm, Focal	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
077	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Joint
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
078	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
078	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
079	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Lymph Node, Draining	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Minimal
	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal consistency, Firm, Focal	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Mild
Spleen	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Minimal
	Abnormal size, Enlarged	Correlated	Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
079	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Liver	Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Ovary	Oviduct	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
080	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Adrenal	No macroscopic observations on tissue		Hypertrophy, Cortex, Present
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Gland, Pituitary	No macroscopic observations on tissue		Cyst, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Lymph Node, Draining	Abnormal size, Enlarged Abnormal size, Enlarged	Correlated Correlated	Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal consistency, Firm, Focal Abnormal consistency, Firm, Focal	Correlated Correlated	Edema, Moderate Inflammation, Moderate
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
080	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart	Kidney
Large Intestine, Cecum	Large Intestine, Colon	Liver	Lung	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
081	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Harderian	No macroscopic observations on tissue		Degeneration/Necrosis, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Moderate
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Site, Injection	Abnormal consistency, Firm, Focal, Site, Injection, 9 Abnormal consistency, Firm, Focal, Site, Injection, 9	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
081	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung
Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary
Oviduct	Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum
Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus	Tongue
Trachea	Ureter	Urinary Bladder	Uterus	Vagina

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
082	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Adrenal	No macroscopic observations on tissue		Tissue Comment: Medulla not in section unilaterally
Gland, Harderian	No macroscopic observations on tissue		Infiltration mononuclear cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal consistency, Firm, Focal Abnormal consistency, Firm, Focal	Correlated Correlated	Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
082	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

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Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
083	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Eye	No macroscopic observations on tissue		Rosettes retina, Minimal
Joint	No macroscopic observations on tissue		Inflammation, Extra-capsular, Minimal
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal
Site, Injection	No macroscopic observations on tissue		Edema, Mild Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

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20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
083	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary	Gland, Parathyroid
Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue	Heart
Kidney	Large Intestine, Cecum	Large Intestine, Colon	Lung	Lymph Node, Mesenteric
Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral	Ovary	Oviduct
Pancreas	Skin	Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum
Spinal Cord	Stomach	Thymus	Tongue	Trachea
Ureter	Urinary Bladder	Uterus	Vagina	

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
084	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Large Intestine, Colon	No macroscopic observations on tissue		Infiltration mixed cell, Mucosa, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	Abnormal consistency, Firm, Focal	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
			Increased cellularity, Hematopoietic cell, Minimal
Ureter	No macroscopic observations on tissue		One-of-pair Missing
			Tissue is unremarkable

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
084	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Liver
Lung	Lymph Node, Draining	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic
Nerve, Peripheral	Ovary	Oviduct	Pancreas	Skin
Small Intestine, Duodenum	Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach
Thymus	Tongue	Trachea	Ureter	Urinary Bladder
Uterus	Vagina			

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
085	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Bone Marrow, Sternum	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal
Gland, Parathyroid	No macroscopic observations on tissue		One-of-pair Missing Tissue is unremarkable
Liver	No macroscopic observations on tissue		Vacuolation, Hepatocyte; Periportal, Minimal
Lymph Node, Draining	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Mild
	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Mild
Lymph Node, Inguinal	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Germinal center, Mild
	Abnormal size, Enlarged, Left	Correlated	Increased cellularity, Plasma cell, Minimal
Site, Injection	Abnormal consistency, Firm, Focal	Correlated	Edema, Mild
	Abnormal consistency, Firm, Focal	Correlated	Inflammation, Mild
Spleen	No macroscopic observations on tissue		Increased cellularity, Hematopoietic cell, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
085	F	3	30 µg /day	17	Dosing	Terminal Euthanasia

The following tissues are unremarkable microscopically:

Artery, Aorta	Bone, Sternum	Brain	Cervix	Esophagus
Eye	Gland, Adrenal	Gland, Harderian	Gland, Lacrimal, Extraorbital	Gland, Mammary
Gland, Parathyroid	Gland, Pituitary	Gland, Salivary	Gland, Thyroid	Gut-Associated Lymphoid Tissue
Heart	Joint	Kidney	Large Intestine, Cecum	Large Intestine, Colon
Lung	Lymph Node, Mesenteric	Muscle, Skeletal	Nerve, Optic	Nerve, Peripheral
Ovary	Oviduct	Pancreas	Skin	Small Intestine, Duodenum
Small Intestine, Ileum	Small Intestine, Jejunum	Spinal Cord	Stomach	Thymus
Tongue	Trachea	Ureter	Urinary Bladder	Uterus
Vagina				

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
086	F	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Joint	No macroscopic observations on tissue		Physeal dysplasia, Minimal
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Liver Lymph Node, Inguinal Spleen

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
087	F	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Infiltration, Macrophage, Mild
Lymph Node, Inguinal	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Minimal
	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Minimal
	Abnormal size, Enlarged	Correlated	Infiltration, Macrophage, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
088	F	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Spleen

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
089	F	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	Abnormal size, Enlarged	Correlated	Increased cellularity, Germinal center, Minimal
	Abnormal size, Enlarged	Correlated	Increased cellularity, Plasma cell, Minimal
	Abnormal size, Enlarged	Correlated	Infiltration, Macrophage, Minimal
Lymph Node, Inguinal	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver

Appendix 11

Individual Macroscopic and Microscopic Observations w/Correlations

20GR142 : 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Animal #	Sex	Group #	Dose	Day of Death	Phase	Status
090	F	3	30 µg /day	22	Recovery	Recovery Euthanasia 1

Tissue	Macroscopic Observations / comments	Status	Microscopic Observations / comments
Lymph Node, Draining	No macroscopic observations on tissue		Increased cellularity, Germinal center, Mild Increased cellularity, Plasma cell, Minimal Infiltration, Macrophage, Mild
Site, Injection	No macroscopic observations on tissue		Inflammation, Minimal
Spleen	No macroscopic observations on tissue		Increased cellularity, Germinal center, Minimal

The following required protocol tissues were not examined microscopically:

No Tissues to list

The following tissues are unremarkable microscopically:

Bone Marrow, Sternum Joint Liver Lymph Node, Inguinal

Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
1	001	ER-0:...	ER-0:...	ER-0:...	-	-
	002	ER-0:...	ER-0:...	ER-0:...	-	-
	003	ER-0:...	ER-0:...	ER-0:...	-	-
	004	ER-0:...	ER-0:...	ER-0:...	-	-
	005	ER-0:...	ER-0:...	ER-0:...	-	-
	006	ER-0:...	ER-0:...	ER-0:...	-	-
	007	ER-0:...	ER-0:...	ER-0:...	-	-
	008	ER-0:...	ER-0:...	ER-0:...	-	-
	009	ER-0:...	ER-0:...	ER-0:...	-	-
	010	ER-0:...	ER-0:...	ER-0:...	-	-
	011	ER-0:...	ER-0:...	ER-0:...	-	-
	012	ER-0:...	ER-0:...	ER-0:...	-	-
	013	ER-0:...	ER-0:...	ER-0:...	-	-
	014	ER-0:...	ER-0:...	ER-0:...	-	-
	015	ER-0:...	ER-0:...	ER-0:...	-	-
2	016	ER-0:...	ER-0:...	ER-0:...	-	-
	017	ER-0:...	ER-0:...	ER-0:...	-	-
	018	ER-0:...	ER-0:...	ER-0:...	-	-
	019	ER-0:...	ER-0:...	ER-0:...	-	-
	020	ER-0:...	ER-0:...	ER-0:...	-	-
	021	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
2	022	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	023	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	024	ER-0:...	ER-0:...	ER-0:...	-	-
	025	ER-0:...	ER-0:...	ER-1:...	-	-
	026	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	027	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	028	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	029	ER-0:...	ER-0:...	ER-0:...	-	-
	030	ER-0:...	ER-0:...	ER-0:...	-	-
3	031	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	032	ER-0:...	ER-0:...	ER-0:...	-	-
	033	ER-0:...	ER-0:...	ER-0:...	-	-
	034	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	035	ER-0:...	ER-0:...	ER-0:...	-	-
	036	ER-0:...	ER-0:...	ER-0:...	-	-
	037	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	038	ER-0:...	ER-0:...	ER-0:...	-	-
	039	ER-0:...	ER-0:...	ER-0:...	-	-
	040	ER-0:...	ER-0:...	ER-0:...	-	-
	041	ER-0:...	ER-0:...	ER-1:...	-	-
	042	ER-0:...	ER-0:...	ER-0:...	-	-

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
3	043	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	044	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	045	ER-0:...	ER-0:...	ER-0:...	ER-1:...	ER-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
1	001	-	-	ER-0:...	ER-0:...	ER-0:...
	002	-	-	ER-0:...	ER-0:...	ER-0:...
	003	-	-	ER-0:...	ER-0:...	ER-0:...
	004	-	-	ER-0:...	ER-0:...	ER-0:...
	005	-	-	ER-0:...	ER-0:...	ER-0:...
	006	-	-	ER-0:...	ER-0:...	ER-0:...
	007	-	-	ER-0:...	ER-0:...	ER-0:...
	008	-	-	ER-0:...	ER-0:...	ER-0:...
	009	-	-	ER-0:...	ER-0:...	ER-0:...
	010	-	-	ER-0:...	ER-0:...	ER-0:...
	011	-	-	ER-0:...	ER-0:...	ER-0:...
	012	-	-	ER-0:...	ER-0:...	ER-0:...
	013	-	-	ER-0:...	ER-0:...	ER-0:...
	014	-	-	ER-0:...	ER-0:...	ER-0:...
	015	-	-	ER-0:...	ER-0:...	ER-0:...
2	016	-	-	ER-0:...	ER-0:...	ER-0:...
	017	-	-	ER-0:...	ER-0:...	ER-0:...
	018	-	-	ER-0:...	ER-0:...	ER-0:...
	019	-	-	ER-0:...	ER-0:...	ER-0:...
	020	-	-	ER-0:...	ER-0:...	ER-0:...
	021	-	-	ER-0:...	ER-0:...	ER-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
2	022	-	-	ER-0:...	ER-0:...	ER-1:...
	023	-	-	ER-0:...	ER-0:...	ER-0:...
	024	-	-	ER-0:...	ER-0:...	ER-1:...
	025	-	-	ER-0:...	ER-0:...	ER-1:...
	026	-	-	ER-0:...	ER-0:...	ER-1:...
	027	-	-	ER-0:...	ER-0:...	ER-1:...
	028	-	-	ER-0:...	ER-0:...	ER-1:...
	029	-	-	ER-0:...	ER-0:...	ER-1:...
	030	-	-	ER-0:...	ER-0:...	ER-0:...
3	031	-	-	ER-0:...	ER-0:...	ER-1:...
	032	-	-	ER-0:...	ER-0:...	ER-1:...
	033	-	-	ER-0:...	ER-0:...	ER-1:...
	034	-	-	ER-0:...	ER-0:...	ER-1:...
	035	-	-	ER-0:...	ER-0:...	ER-1:...
	036	-	-	ER-0:...	ER-0:...	ER-1:...
	037	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-1:...
	038	-	-	ER-0:...	ER-0:...	ER-1:...
	039	-	-	ER-0:...	ER-0:...	ER-0:...
	040	-	-	ER-0:...	ER-0:...	ER-1:...
	041	-	-	ER-0:...	ER-0:...	ER-1:...
	042	-	-	ER-0:...	ER-0:...	ER-1:...

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StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
3	043	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	044	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	045	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-1:...

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
1	001	-	-	-	-	ER-0:...
	002	-	-	-	-	ER-0:...
	003	-	-	-	-	ER-0:...
	004	-	-	-	-	ER-0:...
	005	-	-	-	-	ER-0:...
	006	-	-	-	-	ER-0:...
	007	-	-	-	-	ER-0:...
	008	-	-	-	-	ER-0:...
	009	-	-	-	-	ER-0:...
	010	-	-	-	-	ER-0:...
	011	-	-	-	-	ER-0:...
	012	-	-	-	-	ER-0:...
	013	-	-	-	-	ER-0:...
	014	-	-	-	-	ER-0:...
	015	-	-	-	-	ER-0:...
2	016	-	-	-	-	ER-0:...
	017	-	-	-	-	ER-0:...
	018	ER-0:...	ER-0:...	-	-	ER-0:...
	019	ER-0:...	ER-0:...	-	-	ER-0:...
	020	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	021	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
2	022	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	023	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	024	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	025	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	026	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	027	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	028	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	029	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	030	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
3	031	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	032	ER-1:...	ER-0:...	-	-	ER-0:...
	033	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	034	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	035	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	036	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	037	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	038	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	039	ER-0:...	ER-0:...	-	-	ER-0:...
	040	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	041	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	042	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
3	043	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	044	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	045	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...

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Erythema Grade-Left(ERL)

Males

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
1	001	ER-0:...	ER-0:...	-	-	-
	002	ER-0:...	ER-0:...	-	-	-
	003	ER-0:...	ER-0:...	-	-	-
	004	ER-0:...	ER-0:...	-	-	-
	005	ER-0:...	ER-0:...	-	-	-
	006	ER-0:...	ER-0:...	-	-	-
	007	ER-0:...	ER-0:...	-	-	-
	008	ER-0:...	ER-0:...	-	-	-
	009	ER-0:...	ER-0:...	-	-	-
	010	ER-0:...	ER-0:...	-	-	-
	011	ER-0:...	ER-0:...	-	-	-
	012	ER-0:...	ER-0:...	-	-	-
	013	ER-0:...	ER-0:...	-	-	-
	014	ER-0:...	ER-0:...	-	-	-
	015	ER-0:...	ER-0:...	-	-	-
2	016	ER-0:...	ER-0:...	ER-0:...	-	-
	017	ER-0:...	ER-0:...	-	-	-
	018	ER-0:...	ER-0:...	ER-0:...	-	-
	019	ER-0:...	ER-0:...	ER-0:...	-	-
	020	ER-0:...	ER-0:...	ER-0:...	-	-
	021	ER-0:...	ER-0:...	ER-0:...	-	-

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Males

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
2	022	ER-0:...	ER-0:...	ER-0:...	-	-
	023	ER-0:...	ER-0:...	ER-0:...	-	-
	024	ER-0:...	ER-0:...	ER-0:...	-	-
	025	ER-0:...	ER-0:...	ER-0:...	-	-
	026	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	027	ER-0:...	ER-0:...	ER-0:...	ER-0:...	-
	028	ER-0:...	ER-0:...	ER-0:...	ER-0:...	-
	029	ER-0:...	ER-0:...	-	-	-
	030	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
3	031	ER-0:...	ER-0:...	ER-1:...	-	-
	032	ER-0:...	ER-0:...	-	-	-
	033	ER-0:...	ER-0:...	ER-1:...	-	-
	034	ER-0:...	ER-0:...	ER-0:...	-	-
	035	ER-0:...	ER-0:...	ER-0:...	-	-
	036	ER-0:...	ER-0:...	ER-0:...	-	-
	037	ER-0:...	ER-0:...	ER-0:...	-	-
	038	ER-0:...	ER-0:...	ER-0:...	-	-
	039	ER-0:...	ER-0:...	ER-0:...	-	-
	040	ER-0:...	ER-0:...	ER-0:...	-	-
	041	ER-1:...	ER-0:...	ER-1:...	ER-0:...	ER-0:...
	042	ER-0:...	ER-0:...	ER-0:...	ER-0:...	-

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Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
3	043	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	044	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	045	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...

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Rat/Wistar Han

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Erythema Grade-Left(ERL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
1	001	-
	002	-
	003	-
	004	-
	005	-
	006	-
	007	-
	008	-
	009	-
	010	-
	011	-
	012	-
	013	-
	014	-
	015	-
2	016	-
	017	-
	018	-
	019	-
	020	-
	021	-

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Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
2	022	-
	023	-
	024	-
	025	-
	026	ER-0:...
	027	-
	028	-
	029	-
	030	ER-0:...
3	031	-
	032	-
	033	-
	034	-
	035	-
	036	-
	037	-
	038	-
	039	-
	040	-
	041	ER-0:...
	042	-

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
3	043	ER-0:...
	044	ER-0:...
	045	ER-0:...

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
1	046	ER-0:...	ER-0:...	ER-0:...	-	-
	047	ER-0:...	ER-0:...	ER-0:...	-	-
	048	ER-0:...	ER-0:...	ER-0:...	-	-
	049	ER-0:...	ER-0:...	ER-0:...	-	-
	050	ER-0:...	ER-0:...	ER-0:...	-	-
	051	ER-0:...	ER-0:...	ER-0:...	-	-
	052	ER-0:...	ER-0:...	ER-0:...	-	-
	053	ER-0:...	ER-0:...	ER-0:...	-	-
	054	ER-0:...	ER-0:...	ER-0:...	-	-
	055	ER-0:...	ER-0:...	ER-0:...	-	-
	056	ER-0:...	ER-0:...	ER-0:...	-	-
	057	ER-0:...	ER-0:...	ER-0:...	-	-
	058	ER-0:...	ER-0:...	ER-0:...	-	-
	059	ER-0:...	ER-0:...	ER-0:...	-	-
	060	ER-0:...	ER-0:...	ER-0:...	-	-
2	061	ER-0:...	ER-0:...	ER-0:...	-	-
	062	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	063	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-0:...
	064	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	065	ER-0:...	ER-0:...	ER-0:...	-	-
	066	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...

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Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
2	067	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	068	ER-0:...	ER-0:...	ER-0:...	-	-
	069	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	070	ER-0:...	ER-0:...	ER-0:...	-	-
	071	ER-0:...	ER-0:...	ER-1:...	-	-
	072	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	073	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	074	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	075	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
3	076	ER-0:...	ER-0:...	ER-1:...	-	-
	077	ER-0:...	ER-0:...	ER-1:...	-	-
	078	ER-0:...	ER-0:...	ER-1:...	-	-
	079	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	080	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	081	ER-0:...	ER-0:...	ER-1:...	-	-
	082	ER-0:...	ER-0:...	ER-1:...	-	-
	083	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	084	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	085	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	086	ER-0:...	ER-0:...	ER-1:...	-	-
	087	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...

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Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
3	088	ER-0:...	ER-0:...	ER-1:...	ER-1:...	ER-1:...
	089	ER-0:...	ER-0:...	ER-1:...	-	-
	090	ER-0:...	ER-0:...	ER-1:...	-	-

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Erythema Grade-Left(ERL)

Females

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
1	046	-	-	ER-0:...	ER-0:...	ER-0:...
	047	-	-	ER-0:...	ER-0:...	ER-0:...
	048	-	-	ER-0:...	ER-0:...	ER-0:...
	049	-	-	ER-0:...	ER-0:...	ER-0:...
	050	-	-	ER-0:...	ER-0:...	ER-0:...
	051	-	-	ER-0:...	ER-0:...	ER-0:...
	052	-	-	ER-0:...	ER-0:...	ER-0:...
	053	-	-	ER-0:...	ER-0:...	ER-0:...
	054	-	-	ER-0:...	ER-0:...	ER-0:...
	055	-	-	ER-0:...	ER-0:...	ER-0:...
	056	-	-	ER-0:...	ER-0:...	ER-0:...
	057	-	-	ER-0:...	ER-0:...	ER-0:...
	058	-	-	ER-0:...	ER-0:...	ER-0:...
	059	-	-	ER-0:...	ER-0:...	ER-0:...
	060	-	-	ER-0:...	ER-0:...	ER-0:...
2	061	-	-	ER-0:...	ER-0:...	ER-1:...
	062	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	063	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-1:...
	064	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	065	-	-	ER-0:...	ER-0:...	ER-1:...
	066	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...

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2	067	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	068	-	-	ER-0:...	ER-0:...	ER-1:...
	069	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	070	-	-	ER-0:...	ER-0:...	ER-1:...
	071	-	-	ER-0:...	ER-0:...	ER-1:...
	072	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	073	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	074	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	075	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
3	076	-	-	ER-0:...	ER-0:...	ER-1:...
	077	-	-	ER-0:...	ER-0:...	ER-1:...
	078	-	-	ER-0:...	ER-0:...	ER-1:...
	079	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	080	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	081	-	-	ER-0:...	ER-0:...	ER-1:...
	082	-	-	ER-0:...	ER-0:...	ER-1:...
	083	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	084	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	085	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	086	-	-	ER-0:...	ER-0:...	ER-1:...
	087	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...

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Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
3	088	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-1:...
	089	-	-	ER-0:...	ER-0:...	ER-1:...
	090	-	-	ER-0:...	ER-0:...	ER-1:...

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Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
1	046	-	-	-	-	ER-0:...
	047	-	-	-	-	ER-0:...
	048	-	-	-	-	ER-0:...
	049	-	-	-	-	ER-0:...
	050	-	-	-	-	ER-0:...
	051	-	-	-	-	ER-0:...
	052	-	-	-	-	ER-0:...
	053	-	-	-	-	ER-0:...
	054	-	-	-	-	ER-0:...
	055	-	-	-	-	ER-0:...
	056	-	-	-	-	ER-0:...
	057	-	-	-	-	ER-0:...
	058	-	-	-	-	ER-0:...
	059	-	-	-	-	ER-0:...
	060	-	-	-	-	ER-0:...
2	061	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	062	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	063	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	064	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	065	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	066	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...

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Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Females

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
2	067	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	068	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	069	ER-1:...	ER-0:...	-	-	ER-0:...
	070	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	071	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	072	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	073	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	074	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	075	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
3	076	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	077	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	078	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	079	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	080	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	081	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	082	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	083	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	084	ER-1:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	085	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...
	086	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	087	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Females

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
3	088	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	089	ER-1:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	090	ER-1:...	ER-1:...	ER-1:...	ER-0:...	ER-0:...

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Females

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
1	046	ER-0:...	ER-0:...	-	-	-
	047	ER-0:...	ER-0:...	-	-	-
	048	ER-0:...	ER-0:...	-	-	-
	049	ER-0:...	ER-0:...	-	-	-
	050	ER-0:...	ER-0:...	-	-	-
	051	ER-0:...	ER-0:...	-	-	-
	052	ER-0:...	ER-0:...	-	-	-
	053	ER-0:...	ER-0:...	-	-	-
	054	ER-0:...	ER-0:...	-	-	-
	055	ER-0:...	ER-0:...	-	-	-
	056	ER-0:...	ER-0:...	-	-	-
	057	ER-0:...	ER-0:...	-	-	-
	058	ER-0:...	ER-0:...	-	-	-
	059	ER-0:...	ER-0:...	-	-	-
	060	ER-0:...	ER-0:...	-	-	-
2	061	ER-0:...	ER-0:...	ER-0:...	-	-
	062	ER-0:...	ER-1:...	ER-1:...	-	-
	063	ER-0:...	ER-1:...	ER-1:...	-	-
	064	ER-0:...	ER-1:...	ER-0:...	-	-
	065	ER-0:...	ER-1:...	ER-0:...	-	-
	066	ER-0:...	ER-1:...	ER-0:...	-	-

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Females

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2	067	ER-0:...	ER-1:...	ER-0:...	-	-
	068	ER-0:...	ER-1:...	ER-0:...	-	-
	069	ER-0:...	ER-1:...	ER-0:...	-	-
	070	ER-0:...	ER-0:...	ER-0:...	-	-
	071	ER-0:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	072	ER-0:...	ER-1:...	ER-0:...	ER-1:...	ER-0:...
	073	ER-0:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	074	ER-0:...	ER-0:...	ER-0:...	ER-0:...	ER-0:...
	075	ER-0:...	ER-1:...	ER-1:...	ER-1:...	ER-0:...
3	076	ER-0:...	ER-1:...	ER-1:...	-	-
	077	ER-0:...	ER-1:...	ER-1:...	-	-
	078	ER-0:...	ER-1:...	ER-1:...	-	-
	079	ER-0:...	ER-1:...	ER-0:...	-	-
	080	ER-0:...	ER-1:...	ER-1:...	-	-
	081	ER-0:...	ER-1:...	ER-1:...	-	-
	082	ER-0:...	ER-1:...	ER-1:...	-	-
	083	ER-0:...	ER-1:...	ER-0:...	-	-
	084	ER-0:...	ER-1:...	ER-1:...	-	-
	085	ER-0:...	ER-1:...	ER-1:...	-	-
	086	ER-0:...	ER-1:...	ER-0:...	ER-0:...	ER-0:...
	087	ER-0:...	ER-1:...	ER-1:...	ER-1:...	ER-0:...

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Females

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3	088	ER-0:...	ER-1:...	ER-1:...	ER-1:...	ER-1:...
	089	ER-0:...	ER-1:...	ER-1:...	ER-1:...	ER-0:...
	090	ER-0:...	ER-1:...	ER-1:...	ER-1:...	ER-0:...

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Left(ERL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
1	046	-
	047	-
	048	-
	049	-
	050	-
	051	-
	052	-
	053	-
	054	-
	055	-
	056	-
	057	-
	058	-
	059	-
	060	-
2	061	-
	062	-
	063	-
	064	-
	065	-
	066	-

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Erythema Grade-Left(ERL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
2	067	-
	068	-
	069	-
	070	-
	071	ER-0:...
	072	ER-0:...
	073	ER-0:...
	074	ER-0:...
	075	ER-0:...
3	076	-
	077	-
	078	-
	079	-
	080	-
	081	-
	082	-
	083	-
	084	-
	085	-
	086	ER-0:...
	087	ER-0:...

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Erythema Grade-Left(ERL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
3	088	ER-0:...
	089	ER-0:...
	090	ER-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Right(ERR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
1	001	ER-0:...	ER-0:...
	002	ER-0:...	ER-0:...
	003	ER-0:...	ER-0:...
	004	ER-0:...	ER-0:...
	005	ER-0:...	ER-0:...
	006	ER-0:...	ER-0:...
	007	ER-0:...	ER-0:...
	008	ER-0:...	-
	009	ER-0:...	ER-0:...
	010	ER-0:...	-
	011	ER-0:...	-
	012	ER-0:...	-
	013	ER-0:...	-
	014	ER-0:...	-
	015	ER-0:...	-
2	016	ER-0:...	-
	017	ER-0:...	-
	018	ER-0:...	-
	019	ER-0:...	-
	020	ER-0:...	-
	021	ER-0:...	-

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Right(ERR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
2	022	ER-0:...	-
	023	ER-0:...	-
	024	ER-0:...	-
	025	ER-0:...	-
	026	ER-0:...	-
	027	ER-0:...	-
	028	ER-0:...	-
	029	ER-0:...	-
	030	ER-0:...	-
3	031	ER-0:...	-
	032	ER-0:...	-
	033	ER-0:...	-
	034	ER-0:...	-
	035	ER-0:...	-
	036	ER-0:...	-
	037	ER-0:...	-
	038	ER-0:...	-
	039	ER-0:...	-
	040	ER-0:...	-
	041	ER-0:...	-
	042	ER-0:...	-

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Repeat Dose Toxicity/Toxicity with Recovery

Erythema Grade-Right(ERR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
3	043	ER-0:...	-
	044	ER-0:...	-
	045	ER-0:...	-

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Erythema Grade-Right(ERR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
1	046	ER-0:...	ER-0:...
	047	ER-0:...	ER-0:...
	048	ER-0:...	ER-0:...
	049	ER-0:...	ER-0:...
	050	ER-0:...	ER-0:...
	051	ER-0:...	ER-0:...
	052	ER-0:...	ER-0:...
	053	ER-0:...	ER-0:...
	054	ER-0:...	ER-0:...
	055	ER-0:...	ER-0:...
	056	ER-0:...	ER-0:...
	057	ER-0:...	ER-0:...
	058	ER-0:...	ER-0:...
	059	ER-0:...	-
	060	ER-0:...	-
2	061	ER-0:...	-
	062	ER-0:...	-
	063	ER-0:...	-
	064	ER-0:...	-
	065	ER-0:...	-
	066	ER-0:...	-

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Erythema Grade-Right(ERR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
2	067	ER-0:...	-
	068	ER-0:...	-
	069	ER-0:...	-
	070	ER-0:...	-
	071	ER-0:...	-
	072	ER-0:...	-
	073	ER-0:...	-
	074	ER-0:...	-
	075	ER-0:...	-
3	076	ER-0:...	-
	077	ER-0:...	-
	078	ER-0:...	-
	079	ER-0:...	-
	080	ER-0:...	-
	081	ER-0:...	-
	082	ER-0:...	-
	083	ER-0:...	-
	084	ER-0:...	-
	085	ER-0:...	-
	086	ER-0:...	-
	087	ER-0:...	-

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Erythema Grade-Right(ERR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
3	088	ER-0:...	-
	089	ER-0:...	-
	090	ER-0:...	-

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Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
1	001	ED-0:...	ED-0:...	ED-0:...	-	-
	002	ED-0:...	ED-0:...	ED-0:...	-	-
	003	ED-0:...	ED-0:...	ED-0:...	-	-
	004	ED-0:...	ED-0:...	ED-0:...	-	-
	005	ED-0:...	ED-0:...	ED-0:...	-	-
	006	ED-0:...	ED-0:...	ED-0:...	-	-
	007	ED-0:...	ED-0:...	ED-0:...	-	-
	008	ED-0:...	ED-0:...	ED-0:...	-	-
	009	ED-0:...	ED-0:...	ED-0:...	-	-
	010	ED-0:...	ED-0:...	ED-0:...	-	-
	011	ED-0:...	ED-0:...	ED-0:...	-	-
	012	ED-0:...	ED-0:...	ED-0:...	-	-
	013	ED-0:...	ED-0:...	ED-0:...	-	-
	014	ED-0:...	ED-0:...	ED-0:...	-	-
	015	ED-0:...	ED-0:...	ED-0:...	-	-
2	016	ED-0:...	ED-0:...	ED-0:...	-	-
	017	ED-0:...	ED-0:...	ED-0:...	-	-
	018	ED-0:...	ED-0:...	ED-0:...	-	-
	019	ED-0:...	ED-0:...	ED-1:...	-	-
	020	ED-0:...	ED-0:...	ED-1:...	-	-
	021	ED-0:...	ED-0:...	ED-2:...	ED-1:...	ED-0:...

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2	022	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	023	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	024	ED-0:...	ED-0:...	ED-0:...	-	-
	025	ED-0:...	ED-0:...	ED-1:...	-	-
	026	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	027	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	028	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	029	ED-0:...	ED-0:...	ED-1:...	-	-
	030	ED-0:...	ED-0:...	ED-1:...	-	-
3	031	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	032	ED-0:...	ED-0:...	ED-0:...	-	-
	033	ED-0:...	ED-0:...	ED-1:...	-	-
	034	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-1:...
	035	ED-0:...	ED-0:...	ED-0:...	-	-
	036	ED-0:...	ED-0:...	ED-1:...	-	-
	037	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	038	ED-0:...	ED-0:...	ED-1:...	-	-
	039	ED-0:...	ED-0:...	ED-1:...	-	-
	040	ED-0:...	ED-0:...	ED-1:...	-	-
	041	ED-0:...	ED-0:...	ED-1:...	-	-
	042	ED-0:...	ED-0:...	ED-1:...	-	-

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
3	043	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	044	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	045	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
1	001	-	-	ED-0:...	ED-0:...	ED-0:...
	002	-	-	ED-0:...	ED-0:...	ED-0:...
	003	-	-	ED-0:...	ED-0:...	ED-0:...
	004	-	-	ED-0:...	ED-0:...	ED-0:...
	005	-	-	ED-0:...	ED-0:...	ED-0:...
	006	-	-	ED-0:...	ED-0:...	ED-0:...
	007	-	-	ED-0:...	ED-0:...	ED-0:...
	008	-	-	ED-0:...	ED-0:...	ED-0:...
	009	-	-	ED-0:...	ED-0:...	ED-0:...
	010	-	-	ED-0:...	ED-0:...	ED-0:...
	011	-	-	ED-0:...	ED-0:...	ED-0:...
	012	-	-	ED-0:...	ED-0:...	ED-0:...
	013	-	-	ED-0:...	ED-0:...	ED-0:...
	014	-	-	ED-0:...	ED-0:...	ED-0:...
	015	-	-	ED-0:...	ED-0:...	ED-0:...
2	016	-	-	ED-0:...	ED-0:...	ED-0:...
	017	-	-	ED-0:...	ED-0:...	ED-0:...
	018	-	-	ED-0:...	ED-0:...	ED-2:...
	019	-	-	ED-0:...	ED-0:...	ED-2:...
	020	-	-	ED-0:...	ED-0:...	ED-2:...
	021	-	-	ED-0:...	ED-0:...	ED-2:...

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Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
2	022	-	-	ED-0:...	ED-0:...	ED-3:...
	023	-	-	ED-0:...	ED-0:...	ED-3:...
	024	-	-	ED-0:...	ED-0:...	ED-3:...
	025	-	-	ED-0:...	ED-0:...	ED-3:...
	026	-	-	ED-0:...	ED-0:...	ED-3:...
	027	-	-	ED-0:...	ED-0:...	ED-2:...
	028	-	-	ED-0:...	ED-0:...	ED-3:...
	029	-	-	ED-0:...	ED-0:...	ED-3:...
	030	-	-	ED-0:...	ED-0:...	ED-2:...
3	031	-	-	ED-0:...	ED-0:...	ED-3:...
	032	-	-	ED-0:...	ED-0:...	ED-2:...
	033	-	-	ED-0:...	ED-0:...	ED-3:...
	034	-	-	ED-0:...	ED-0:...	ED-2:...
	035	-	-	ED-0:...	ED-0:...	ED-3:...
	036	-	-	ED-0:...	ED-0:...	ED-2:...
	037	ED-2:...	ED-1:...	ED-0:...	ED-0:...	ED-3:...
	038	-	-	ED-0:...	ED-0:...	ED-3:...
	039	-	-	ED-0:...	ED-0:...	ED-2:...
	040	-	-	ED-0:...	ED-0:...	ED-3:...
	041	-	-	ED-0:...	ED-0:...	ED-3:...
	042	-	-	ED-0:...	ED-0:...	ED-3:...

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Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
3	043	ED-2:...	ED-1:...	ED-0:...	ED-0:...	ED-3:...
	044	ED-2:...	ED-1:...	ED-0:...	ED-0:...	ED-3:...
	045	ED-2:...	ED-1:...	ED-0:...	ED-0:...	ED-3:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
1	001	-	-	-	-	ED-0:...
	002	-	-	-	-	ED-0:...
	003	-	-	-	-	ED-0:...
	004	-	-	-	-	ED-0:...
	005	-	-	-	-	ED-0:...
	006	-	-	-	-	ED-0:...
	007	-	-	-	-	ED-0:...
	008	-	-	-	-	ED-0:...
	009	-	-	-	-	ED-0:...
	010	-	-	-	-	ED-0:...
	011	-	-	-	-	ED-0:...
	012	-	-	-	-	ED-0:...
	013	-	-	-	-	ED-0:...
	014	-	-	-	-	ED-0:...
	015	-	-	-	-	ED-0:...
2	016	-	-	-	-	ED-0:...
	017	-	-	-	-	ED-0:...
	018	ED-2:...	ED-1:...	-	-	ED-0:...
	019	ED-2:...	ED-1:...	-	-	ED-0:...
	020	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	021	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
2	022	ED-3:...	ED-2:...	ED-1:...	ED-1:...	ED-0:...
	023	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	024	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	025	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	026	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	027	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	028	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	029	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	030	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
3	031	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	032	ED-2:...	ED-1:...	-	-	ED-0:...
	033	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	034	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	035	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	036	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	037	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	038	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	039	ED-2:...	ED-1:...	-	-	ED-0:...
	040	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	041	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	042	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...

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Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
3	043	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	044	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	045	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
1	001	ED-0:...	ED-0:...	-	-	-
	002	ED-0:...	ED-0:...	-	-	-
	003	ED-0:...	ED-0:...	-	-	-
	004	ED-0:...	ED-0:...	-	-	-
	005	ED-0:...	ED-0:...	-	-	-
	006	ED-0:...	ED-0:...	-	-	-
	007	ED-0:...	ED-0:...	-	-	-
	008	ED-0:...	ED-0:...	-	-	-
	009	ED-0:...	ED-0:...	-	-	-
	010	ED-0:...	ED-0:...	-	-	-
	011	ED-0:...	ED-0:...	-	-	-
	012	ED-0:...	ED-0:...	-	-	-
	013	ED-0:...	ED-0:...	-	-	-
	014	ED-0:...	ED-0:...	-	-	-
	015	ED-0:...	ED-0:...	-	-	-
2	016	ED-0:...	ED-2:...	ED-2:...	-	-
	017	ED-0:...	ED-0:...	-	-	-
	018	ED-0:...	ED-2:...	ED-2:...	-	-
	019	ED-0:...	ED-2:...	ED-2:...	-	-
	020	ED-0:...	ED-2:...	ED-2:...	-	-
	021	ED-0:...	ED-2:...	ED-2:...	-	-

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Males

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
2	022	ED-0:...	ED-2:...	ED-2:...	-	-
	023	ED-0:...	ED-2:...	ED-2:...	-	-
	024	ED-0:...	ED-2:...	ED-2:...	-	-
	025	ED-0:...	ED-2:...	ED-2:...	-	-
	026	ED-1:...	ED-2:...	ED-2:...	ED-2:...	ED-0:...
	027	ED-0:...	ED-3:...	ED-3:...	ED-1:...	-
	028	ED-0:...	ED-3:...	ED-3:...	ED-1:...	-
	029	ED-1:...	ED-1:...	-	-	-
	030	ED-0:...	ED-2:...	ED-2:...	ED-2:...	ED-1:...
3	031	ED-0:...	ED-2:...	ED-3:...	-	-
	032	ED-0:...	ED-1:...	-	-	-
	033	ED-0:...	ED-2:...	ED-2:...	-	-
	034	ED-0:...	ED-2:...	ED-2:...	-	-
	035	ED-0:...	ED-2:...	ED-2:...	-	-
	036	ED-1:...	ED-2:...	ED-2:...	-	-
	037	ED-1:...	ED-2:...	ED-2:...	-	-
	038	ED-1:...	ED-2:...	ED-2:...	-	-
	039	ED-0:...	ED-2:...	ED-2:...	-	-
	040	ED-0:...	ED-2:...	ED-2:...	-	-
	041	ED-1:...	ED-3:...	ED-3:...	ED-2:...	ED-0:...
	042	ED-0:...	ED-2:...	ED-2:...	ED-1:...	-

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
3	043	ED-0:...	ED-3:...	ED-3:...	ED-2:...	ED-0:...
	044	ED-1:...	ED-3:...	ED-3:...	ED-2:...	ED-0:...
	045	ED-0:...	ED-2:...	ED-2:...	ED-2:...	ED-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
1	001	-
	002	-
	003	-
	004	-
	005	-
	006	-
	007	-
	008	-
	009	-
	010	-
	011	-
	012	-
	013	-
	014	-
	015	-
2	016	-
	017	-
	018	-
	019	-
	020	-
	021	-

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Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
2	022	-
	023	-
	024	-
	025	-
	026	ED-1:...
	027	-
	028	-
	029	-
	030	ED-1:...
3	031	-
	032	-
	033	-
	034	-
	035	-
	036	-
	037	-
	038	-
	039	-
	040	-
	041	ED-1:...
	042	-

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Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Males

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
3	043	ED-0:...
	044	ED-0:...
	045	ED-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
1	046	ED-0:...	ED-0:...	ED-0:...	-	-
	047	ED-0:...	ED-0:...	ED-0:...	-	-
	048	ED-0:...	ED-0:...	ED-0:...	-	-
	049	ED-0:...	ED-0:...	ED-0:...	-	-
	050	ED-0:...	ED-0:...	ED-0:...	-	-
	051	ED-0:...	ED-0:...	ED-0:...	-	-
	052	ED-0:...	ED-0:...	ED-0:...	-	-
	053	ED-0:...	ED-0:...	ED-0:...	-	-
	054	ED-0:...	ED-0:...	ED-0:...	-	-
	055	ED-0:...	ED-0:...	ED-0:...	-	-
	056	ED-0:...	ED-0:...	ED-0:...	-	-
	057	ED-0:...	ED-0:...	ED-0:...	-	-
	058	ED-0:...	ED-0:...	ED-0:...	-	-
	059	ED-0:...	ED-0:...	ED-0:...	-	-
	060	ED-0:...	ED-0:...	ED-0:...	-	-
2	061	ED-0:...	ED-0:...	ED-1:...	-	-
	062	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	063	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	064	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	065	ED-0:...	ED-0:...	ED-1:...	-	-
	066	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
2	067	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	068	ED-0:...	ED-0:...	ED-1:...	-	-
	069	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	070	ED-0:...	ED-0:...	ED-1:...	-	-
	071	ED-0:...	ED-0:...	ED-1:...	-	-
	072	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	073	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	074	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	075	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
3	076	ED-0:...	ED-0:...	ED-1:...	-	-
	077	ED-0:...	ED-0:...	ED-1:...	-	-
	078	ED-0:...	ED-0:...	ED-1:...	-	-
	079	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	080	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	081	ED-0:...	ED-0:...	ED-1:...	-	-
	082	ED-0:...	ED-0:...	ED-1:...	-	-
	083	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	084	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	085	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	086	ED-0:...	ED-0:...	ED-1:...	-	-
	087	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...

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Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 3 Session: S4-48 HPD	Day: 4 Session: S5-72 HPD
3	088	ED-0:...	ED-0:...	ED-2:...	ED-2:...	ED-2:...
	089	ED-0:...	ED-0:...	ED-1:...	-	-
	090	ED-0:...	ED-1:...	ED-1:...	-	-

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
1	046	-	-	ED-0:...	ED-0:...	ED-0:...
	047	-	-	ED-0:...	ED-0:...	ED-0:...
	048	-	-	ED-0:...	ED-0:...	ED-0:...
	049	-	-	ED-0:...	ED-0:...	ED-0:...
	050	-	-	ED-0:...	ED-0:...	ED-0:...
	051	-	-	ED-0:...	ED-0:...	ED-0:...
	052	-	-	ED-0:...	ED-0:...	ED-0:...
	053	-	-	ED-0:...	ED-0:...	ED-0:...
	054	-	-	ED-0:...	ED-0:...	ED-0:...
	055	-	-	ED-0:...	ED-0:...	ED-0:...
	056	-	-	ED-0:...	ED-0:...	ED-0:...
	057	-	-	ED-0:...	ED-0:...	ED-0:...
	058	-	-	ED-0:...	ED-0:...	ED-0:...
	059	-	-	ED-0:...	ED-0:...	ED-0:...
	060	-	-	ED-0:...	ED-0:...	ED-0:...
2	061	-	-	ED-0:...	ED-0:...	ED-2:...
	062	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	063	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	064	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	065	-	-	ED-0:...	ED-0:...	ED-3:...
	066	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...

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2	067	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	068	-	-	ED-0:...	ED-0:...	ED-3:...
	069	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	070	-	-	ED-0:...	ED-0:...	ED-3:...
	071	-	-	ED-0:...	ED-0:...	ED-3:...
	072	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	073	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	074	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	075	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
3	076	-	-	ED-0:...	ED-0:...	ED-3:...
	077	-	-	ED-0:...	ED-0:...	ED-3:...
	078	-	-	ED-0:...	ED-0:...	ED-3:...
	079	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	080	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	081	-	-	ED-0:...	ED-0:...	ED-2:...
	082	-	-	ED-0:...	ED-0:...	ED-3:...
	083	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	084	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	085	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...
	086	-	-	ED-0:...	ED-0:...	ED-2:...
	087	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-3:...

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 6 Session: S6-120 HPD	Day: 7 Session: S7-144 HPD	Day: 8 Session: S1-Predose	Session: S2-4 HPD	Day: 9 Session: S3-24 HPD
3	088	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-2:...
	089	-	-	ED-0:...	ED-0:...	ED-2:...
	090	-	-	ED-0:...	ED-0:...	ED-2:...

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
1	046	-	-	-	-	ED-0:...
	047	-	-	-	-	ED-0:...
	048	-	-	-	-	ED-0:...
	049	-	-	-	-	ED-0:...
	050	-	-	-	-	ED-0:...
	051	-	-	-	-	ED-0:...
	052	-	-	-	-	ED-0:...
	053	-	-	-	-	ED-0:...
	054	-	-	-	-	ED-0:...
	055	-	-	-	-	ED-0:...
	056	-	-	-	-	ED-0:...
	057	-	-	-	-	ED-0:...
	058	-	-	-	-	ED-0:...
	059	-	-	-	-	ED-0:...
	060	-	-	-	-	ED-0:...
2	061	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	062	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	063	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	064	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	065	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	066	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...

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Rat/Wistar Han

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2	067	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	068	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	069	ED-2:...	ED-1:...	-	-	ED-0:...
	070	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	071	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	072	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	073	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	074	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	075	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
3	076	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	077	ED-3:...	ED-3:...	ED-2:...	ED-0:...	ED-0:...
	078	ED-3:...	ED-3:...	ED-2:...	ED-1:...	ED-0:...
	079	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	080	ED-3:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	081	ED-3:...	ED-2:...	ED-0:...	ED-0:...	ED-0:...
	082	ED-3:...	ED-2:...	ED-0:...	ED-0:...	ED-0:...
	083	ED-3:...	ED-3:...	ED-0:...	ED-0:...	ED-0:...
	084	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	085	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	086	ED-2:...	ED-2:...	ED-1:...	ED-0:...	ED-0:...
	087	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...

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Dermal Assessment Left/Right Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Dosing Day: 10 Session: S4-48 HPD	Day: 11 Session: S5-72 HPD	Day: 13 Session: S6-120 HPD	Day: 14 Session: S7-144 HPD	Day: 15 Session: S1-Predose
3	088	ED-3:...	ED-3:...	ED-1:...	ED-0:...	ED-0:...
	089	ED-2:...	ED-2:...	ED-0:...	ED-0:...	ED-0:...
	090	ED-2:...	ED-2:...	ED-2:...	ED-0:...	ED-0:...

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Females

Group #	Animal #	Dosing Day: 15 Session: S2-4 HPD	Day: 16 Session: S3-24 HPD	Day: 17 Session: S4-48 HPD	Recovery Day: 1 Session: S5-72 HPD	Day: 3 Session: S6-120 HPD
1	046	ED-0:...	ED-0:...	-	-	-
	047	ED-0:...	ED-0:...	-	-	-
	048	ED-0:...	ED-0:...	-	-	-
	049	ED-0:...	ED-0:...	-	-	-
	050	ED-0:...	ED-0:...	-	-	-
	051	ED-0:...	ED-0:...	-	-	-
	052	ED-0:...	ED-0:...	-	-	-
	053	ED-0:...	ED-0:...	-	-	-
	054	ED-0:...	ED-0:...	-	-	-
	055	ED-0:...	ED-0:...	-	-	-
	056	ED-0:...	ED-0:...	-	-	-
	057	ED-0:...	ED-0:...	-	-	-
	058	ED-0:...	ED-0:...	-	-	-
	059	ED-0:...	ED-0:...	-	-	-
	060	ED-0:...	ED-0:...	-	-	-
2	061	ED-0:...	ED-2:...	ED-2:...	-	-
	062	ED-0:...	ED-3:...	ED-3:...	-	-
	063	ED-0:...	ED-3:...	ED-3:...	-	-
	064	ED-0:...	ED-3:...	ED-2:...	-	-
	065	ED-0:...	ED-2:...	ED-2:...	-	-
	066	ED-0:...	ED-3:...	ED-3:...	-	-

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2	067	ED-0:...	ED-3:...	ED-2:...	-	-
	068	ED-0:...	ED-2:...	ED-2:...	-	-
	069	ED-0:...	ED-3:...	ED-2:...	-	-
	070	ED-0:...	ED-2:...	ED-2:...	-	-
	071	ED-0:...	ED-3:...	ED-3:...	ED-2:...	ED-1:...
	072	ED-0:...	ED-3:...	ED-3:...	ED-3:...	ED-1:...
	073	ED-0:...	ED-2:...	ED-2:...	ED-2:...	ED-1:...
	074	ED-0:...	ED-2:...	ED-1:...	ED-2:...	ED-1:...
	075	ED-0:...	ED-3:...	ED-3:...	ED-3:...	ED-0:...
3	076	ED-0:...	ED-3:...	ED-3:...	-	-
	077	ED-0:...	ED-3:...	ED-3:...	-	-
	078	ED-0:...	ED-3:...	ED-3:...	-	-
	079	ED-0:...	ED-3:...	ED-2:...	-	-
	080	ED-0:...	ED-3:...	ED-3:...	-	-
	081	ED-0:...	ED-3:...	ED-3:...	-	-
	082	ED-0:...	ED-3:...	ED-2:...	-	-
	083	ED-0:...	ED-3:...	ED-3:...	-	-
	084	ED-0:...	ED-3:...	ED-2:...	-	-
	085	ED-0:...	ED-2:...	ED-3:...	-	-
	086	ED-0:...	ED-2:...	ED-2:...	ED-2:...	ED-1:...
	087	ED-0:...	ED-3:...	ED-3:...	ED-3:...	ED-1:...

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3	088	ED-0:...	ED-3:...	ED-3:...	ED-3:...	ED-1:...
	089	ED-0:...	ED-2:...	ED-2:...	ED-3:...	ED-0:...
	090	ED-0:...	ED-2:...	ED-2:...	ED-2:...	ED-1:...

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Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
1	046	-
	047	-
	048	-
	049	-
	050	-
	051	-
	052	-
	053	-
	054	-
	055	-
	056	-
	057	-
	058	-
	059	-
	060	-
2	061	-
	062	-
	063	-
	064	-
	065	-
	066	-

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
2	067	-
	068	-
	069	-
	070	-
	071	ED-0:...
	072	ED-0:...
	073	ED-0:...
	074	ED-0:...
	075	ED-0:...
3	076	-
	077	-
	078	-
	079	-
	080	-
	081	-
	082	-
	083	-
	084	-
	085	-
	086	ED-0:...
	087	ED-0:...

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Left(EDL)

Females

Group #	Animal #	Recovery Day: 4 Session: S7-144 HPD
3	088	ED-0:...
	089	ED-0:...
	090	ED-0:...

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Study: 20GR142
StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY
Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
1	001	ED-0:...	ED-0:...
	002	ED-0:...	ED-0:...
	003	ED-0:...	ED-0:...
	004	ED-0:...	ED-0:...
	005	ED-0:...	ED-0:...
	006	ED-0:...	ED-0:...
	007	ED-0:...	ED-0:...
	008	ED-0:...	-
	009	ED-0:...	ED-0:...
	010	ED-0:...	-
	011	ED-0:...	-
	012	ED-0:...	-
	013	ED-0:...	-
	014	ED-0:...	-
	015	ED-0:...	-
2	016	ED-0:...	-
	017	ED-0:...	-
	018	ED-0:...	-
	019	ED-0:...	-
	020	ED-0:...	-
	021	ED-0:...	-

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Dermal Assessment Left/Right Report with Individual Values

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Pfizer

Pristima® Version 7.4.3 Build 25

Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
2	022	ED-0:...	-
	023	ED-0:...	-
	024	ED-0:...	-
	025	ED-0:...	-
	026	ED-0:...	-
	027	ED-0:...	-
	028	ED-0:...	-
	029	ED-0:...	-
	030	ED-0:...	-
3	031	ED-0:...	-
	032	ED-0:...	-
	033	ED-0:...	-
	034	ED-0:...	-
	035	ED-0:...	-
	036	ED-0:...	-
	037	ED-0:...	-
	038	ED-0:...	-
	039	ED-0:...	-
	040	ED-0:...	-
	041	ED-0:...	-
	042	ED-0:...	-

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Males

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
3	043	ED-0:...	-
	044	ED-0:...	-
	045	ED-0:...	-

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
1	046	ED-0:...	ED-0:...
	047	ED-0:...	ED-0:...
	048	ED-0:...	ED-0:...
	049	ED-0:...	ED-0:...
	050	ED-0:...	ED-0:...
	051	ED-0:...	ED-0:...
	052	ED-0:...	ED-0:...
	053	ED-0:...	ED-0:...
	054	ED-0:...	ED-0:...
	055	ED-0:...	ED-0:...
	056	ED-0:...	ED-0:...
	057	ED-0:...	ED-0:...
	058	ED-0:...	ED-0:...
	059	ED-0:...	-
	060	ED-0:...	-
2	061	ED-0:...	-
	062	ED-0:...	-
	063	ED-0:...	-
	064	ED-0:...	-
	065	ED-0:...	-
	066	ED-0:...	-

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
2	067	ED-0:...	-
	068	ED-0:...	-
	069	ED-0:...	-
	070	ED-0:...	-
	071	ED-0:...	-
	072	ED-0:...	-
	073	ED-0:...	-
	074	ED-0:...	-
	075	ED-0:...	-
3	076	ED-0:...	-
	077	ED-0:...	-
	078	ED-0:...	-
	079	ED-0:...	-
	080	ED-0:...	-
	081	ED-0:...	-
	082	ED-0:...	-
	083	ED-0:...	-
	084	ED-0:...	-
	085	ED-0:...	-
	086	ED-0:...	-
	087	ED-0:...	-

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Edema Grade-Right(EDR)

Females

Group #	Animal #	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD
3	088	ED-0:...	-
	089	ED-0:...	-
	090	ED-0:...	-

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Body Temperature Report with Individual Values

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Pfizer

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
1	001	36.8	36.1	35.8	37.4	37.7
	002	36.2	35.9	36.0	38.8	37.7
	003	36.9	36.5	36.1	38.2	36.7
	004	36.0	37.8	36.4	38.7	36.9
	005	37.1	36.8	35.6	38.3	37.7
	006	36.5	37.1	36.8	38.1	36.7
	007	36.0	37.2	36.4	38.5	37.4
	008	36.9	37.8	35.6	38.1	37.6
	009	36.2	37.0	36.5	38.8	37.6
	010	37.5	37.2	35.6	38.2	36.9
	011	36.7	37.1	36.2	38.3	37.5
	012	36.5	36.9	35.2	38.3	36.6
	013	35.6	36.8	36.2	38.2	37.9
	014	37.8	37.1	35.2	38.5	37.7
	015	37.7	37.1	36.0	38.2	37.3
2	016	36.4	37.0	36.1	38.3	36.7
	017	36.8	36.9	36.1	39.2	37.1
	018	37.5	37.0	36.3	39.2	37.9
	019	36.0	36.8	37.0	38.7	36.9
	020	36.1	37.1	35.9	38.5	36.7
	021	36.0	36.9	35.9	39.3	36.9

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Body Temperature Report with Individual Values

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Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
2	022	36.9	37.1	35.5	39.1	37.7
	023	36.6	37.1	35.8	38.4	36.9
	024	37.4	36.9	35.0	38.5	37.3
	025	37.5	38.5	35.1	39.1	38.4
	026	36.1	36.9	36.9	39.1	36.6
	027	37.7	36.8	36.7	38.8	36.9
	028	37.7	37.0	35.5	38.9	37.4
	029	36.3	37.1	36.6	38.4	36.8
	030	35.2	36.8	35.5	39.3	38.5
3	031	38.2	36.8	36.3	38.8	36.4
	032	36.3	37.0	36.1	39.4	36.6
	033	37.0	36.9	36.4	38.9	37.0
	034	37.9	36.9	35.6	38.6	36.4
	035	38.2	37.1	35.3	38.4	37.9
	036	36.8	37.2	36.2	38.7	37.1
	037	37.0	36.7	36.0	39.0	37.0
	038	36.2	37.0	35.7	39.2	37.6
	039	36.8	36.8	36.0	39.1	36.8
	040	36.0	37.4	36.2	39.0	37.6
	041	35.9	36.9	36.4	38.6	37.6
	042	36.0	36.7	36.4	38.8	37.4

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Study: 20GR142

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
3	043	36.4	37.2	36.6	39.5	37.3
	044	36.3	37.0	35.9	39.8	38.0
	045	37.0	37.1	36.1	39.5	37.4

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
1	001	34.6	37.0	37.6	36.8	37.3
	002	34.5	36.9	37.4	36.5	36.7
	003	34.7	37.1	37.8	37.2	37.2
	004	34.9	36.9	38.8	36.8	37.1
	005	36.3	37.0	38.0	37.7	36.9
	006	36.4	37.1	37.3	37.0	37.0
	007	35.5	36.9	37.4	36.8	37.1
	008	35.5	36.6	37.9	37.3	37.1
	009	36.2	36.3	37.9	36.9	37.3
	010	37.2	36.5	37.4	37.4	36.7
	011	36.4	36.8	38.6	37.0	37.0
	012	36.6	37.4	38.2	37.8	37.4
	013	36.8	37.6	37.6	37.5	37.0
	014	36.9	37.6	38.2	37.7	36.3
	015	37.2	37.6	37.9	38.0	36.7
2	016	36.0	39.0	37.4	37.5	38.6
	017	36.1	38.4	37.8	38.1	38.5
	018	36.0	37.5	38.5	38.8	38.4
	019	36.7	37.9	37.8	38.5	37.6
	020	37.2	38.1	37.2	37.9	37.5
	021	36.4	38.3	37.6	38.1	38.9

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Body Temperature Report with Individual Values

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
2	022	35.1	37.8	37.9	38.4	38.5
	023	35.9	37.1	37.2	37.9	37.5
	024	36.1	39.0	38.2	38.0	38.8
	025	36.4	39.2	38.7	38.8	38.4
	026	37.3	37.4	36.6	37.7	37.7
	027	37.4	37.7	38.3	37.6	37.4
	028	37.2	37.6	37.0	37.9	38.3
	029	37.4	37.9	37.5	37.5	38.5
	030	37.8	37.9	38.4	37.9	38.3
3	031	36.7	38.6	37.3	38.5	38.5
	032	35.8	38.4	37.1	37.8	38.0
	033	36.7	38.4	37.1	38.2	38.0
	034	36.2	38.6	36.8	38.0	38.2
	035	37.0	38.5	38.1	38.8	39.1
	036	36.3	38.6	36.8	37.6	38.5
	037	37.4	38.8	37.1	37.6	38.5
	038	36.2	38.4	37.8	37.8	38.6
	039	36.2	38.6	37.0	37.8	38.0
	040	38.1	38.4	38.8	38.5	39.0
	041	36.1	37.9	37.7	38.2	37.8
	042	36.9	37.0	38.7	38.1	38.4

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Males

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
3	043	36.9	38.4	37.4	38.5	38.7
	044	36.6	38.3	37.6	37.5	38.7
	045	36.2	38.0	37.7	37.2	37.9

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Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Females

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
1	046	38.3	37.4	37.0	37.7	38.2
	047	37.1	37.8	38.6	37.3	37.7
	048	38.7	38.0	38.2	37.7	37.9
	049	38.0	36.8	37.8	38.0	37.7
	050	38.0	38.0	38.2	37.5	38.0
	051	37.5	38.6	38.7	38.2	38.3
	052	38.5	37.6	38.2	37.8	37.8
	053	37.8	37.5	38.8	38.0	37.9
	054	37.7	38.4	37.6	37.6	38.8
	055	36.8	37.2	38.5	37.9	37.9
	056	38.5	37.0	37.7	37.0	38.0
	057	37.8	37.8	38.0	37.0	37.6
	058	37.8	35.9	36.9	37.3	38.0
	059	36.5	37.9	37.7	36.7	37.5
	060	37.2	37.7	38.0	37.5	37.8
2	061	36.2	36.9	37.8	37.6	37.9
	062	37.4	38.3	38.5	38.1	37.6
	063	37.4	37.4	38.2	37.5	38.0
	064	36.9	36.4	39.4	36.6	37.9
	065	38.5	38.3	38.1	38.9	37.7
	066	36.6	38.2	37.9	37.1	38.1

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Females

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
2	067	36.8	38.4	38.7	37.8	38.3
	068	38.1	37.7	38.7	37.3	38.0
	069	37.8	36.7	39.0	39.0	37.7
	070	36.6	37.9	38.7	37.1	37.8
	071	37.3	37.1	37.1	37.8	37.6
	072	36.3	36.6	37.8	37.9	37.8
	073	38.1	37.4	39.3	38.2	38.2
	074	37.3	36.4	38.1	37.5	37.9
	075	36.6	38.3	38.6	38.0	37.5
3	076	38.5	37.9	38.7	38.2	37.7
	077	37.7	36.9	38.8	38.0	38.0
	078	37.6	38.3	38.6	38.1	37.9
	079	37.6	38.0	37.6	38.1	37.9
	080	38.1	38.8	38.5	37.7	38.0
	081	38.0	36.6	38.5	37.8	37.8
	082	38.8	37.6	39.0	38.7	37.7
	083	37.4	37.9	38.1	37.5	38.1
	084	36.5	38.2	39.1	38.4	37.7
	085	38.2	37.6	38.9	37.9	37.6
	086	38.6	37.6	37.9	37.9	38.0
	087	36.8	38.0	39.0	38.9	39.0

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Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Females

Group #	Animal #	PID Day: 6 Session: S1-Predose	Dosing Day: 1 Session: S1-Predose	Session: S2-4 HPD	Day: 2 Session: S3-24 HPD	Day: 8 Session: S1-Predose
3	088	38.6	38.8	38.7	37.8	37.9
	089	38.0	38.6	38.5	37.9	37.9
	090	36.1	37.9	38.3	37.9	38.1

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Body Temp(BT)-(°C)

Females

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
1	046	37.4	37.1	36.8	36.8	37.0
	047	38.0	37.0	37.3	37.0	37.7
	048	37.3	36.6	37.5	37.7	37.0
	049	37.0	37.8	37.0	37.8	37.7
	050	37.3	37.3	37.3	37.1	36.8
	051	37.9	38.3	37.1	39.0	37.3
	052	37.5	37.5	37.8	37.8	36.9
	053	38.0	37.7	37.1	38.3	37.1
	054	37.7	37.9	36.9	39.0	37.3
	055	37.7	37.4	37.2	36.5	36.7
	056	37.9	37.1	37.0	38.3	37.4
	057	38.7	37.1	37.3	39.1	37.5
	058	37.7	36.8	37.7	38.4	37.0
	059	36.9	38.0	37.2	38.5	37.5
	060	37.1	37.7	36.9	37.9	37.1
2	061	37.2	38.2	38.0	37.5	37.7
	062	38.0	38.6	38.1	38.9	37.7
	063	37.8	37.4	37.5	38.5	38.1
	064	37.8	37.9	38.1	39.2	37.6
	065	37.6	37.9	38.3	37.8	37.7
	066	37.5	38.5	37.1	37.6	37.0

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Body Temperature Report with Individual Values

Printed: 23 Jul 2020 04:35:42 PM

Pfizer

Pristima® Version 7.4.3 Build 25

Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Females

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
2	067	37.6	38.9	38.3	38.8	37.9
	068	37.5	38.6	37.7	37.2	37.8
	069	39.2	38.1	37.5	38.2	37.8
	070	38.5	38.9	38.3	38.0	38.5
	071	37.8	38.5	37.8	37.3	37.3
	072	37.9	38.9	37.9	37.7	37.6
	073	37.9	37.8	38.1	38.1	37.4
	074	37.4	38.7	38.5	37.8	37.6
	075	37.9	38.5	38.7	38.3	37.7
3	076	38.1	38.9	38.2	38.3	37.0
	077	39.0	38.0	38.1	37.5	38.0
	078	38.3	38.9	37.7	38.5	37.9
	079	38.7	38.5	38.4	37.2	38.1
	080	37.7	38.9	37.8	37.0	38.3
	081	38.3	38.0	37.8	37.9	37.9
	082	38.1	39.3	38.3	38.6	38.2
	083	38.2	38.9	38.0	37.0	37.9
	084	37.8	38.5	37.9	38.4	37.8
	085	38.1	38.8	38.0	38.6	37.3
	086	38.4	38.9	37.7	38.6	37.8
	087	39.4	38.9	38.0	38.7	37.9

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

Body Temperature Report with Individual Values

Printed: 23 Jul 2020 04:35:42 PM

Pfizer

Pristima® Version 7.4.3 Build 25

Study: 20GR142

StudyTitle: 17-DAY INTRAMUSCULAR TOXICITY STUDY IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Test Article: BNT162b2(V9), BNT162b3c

Rat/Wistar Han

Repeat Dose Toxicity/Toxicity with Recovery

Body Temp(BT)-(°C)

Females

Group #	Animal #	Dosing Day: 8 Session: S2-4 HPD	Day: 9 Session: S3-24 HPD	Day: 15 Session: S1-Predose	Session: S2-4 HPD	Day: 16 Session: S3-24 HPD
3	088	38.0	38.0	38.2	38.8	37.7
	089	38.2	38.1	37.9	38.3	38.6
	090	37.9	38.3	38.1	38.0	37.2

S = Scheduled Animal Room, U = UnScheduled Animal Room, N = Scheduled Necropsy, n = UnScheduled Necropsy

C O N F I D E N T I A L

OPHTHALMOLOGY REPORT:

17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Testing Facility Study Number: 20GR142

Alternative Test Article Identifier(s):

PF-07302048: Generic number for COVID-19 vaccine program

BNT162b2 (V9): BNT162b2 (Version 9); RBP020.2; PF-07305885

BNT162b3c: BNT162b3; RBP020.8; PF-07315256

TESTING FACILITY:
Pfizer
Drug Safety Research & Development
Eastern Point Road
Groton, CT 06340 USA

SIGNATURES

I confirm that this report accurately reflects my interpretation of the ophthalmology data.

(b) (6)
Clinical Veterinarian
Ophthalmology

For signatures see the Document Approval Record.

GLP COMPLIANCE STATEMENT

This portion of the study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58).

Ophthalmology Report for Study 20GR142

TABLE OF CONTENTS

1. INTRODUCTION AND OBJECTIVE

BNT162b2 (Version 9 [V9]) and BNT162b3c are candidate COVID-19 vaccines, which are based on an RNA platform and express the SARS-CoV-2 spike protein or its derivatives. The objective of this study was to determine the toxicity and development of a specific immune response to the antigens in each of the vaccine candidates following administration of intramuscular (IM) doses once weekly for a total of 3 doses to Wistar Han (Crl:WI[Han]) rats. The reversibility of effects was evaluated following a 3-week recovery phase.

Group designations and doses are indicated in the table below.

Group Number	Test Article or Vehicle Dose (µg RNA)/Dose Day	Dose Volume (µL/injection site)[a]	Animal Numbers	
			Males	Females
1	0[b]	60	1-15	46-60
2	30[c]	60	16-30	61-75
3	30[d]	60	31-45	76-90

a. Each animal received a single intramuscular injection on each dose day.
b. Sterile saline.
c. BNT162b2 (V9).
d. BNT162b3c.

Doses were administered by a single intramuscular injection (60 µL) on each dosing day (Days 1, 8, and 15) into the left hindlimb quadriceps muscle.

The first 10 animals/sex/group, by ascending animal order, were designated for necropsy at the end of the dosing phase. The remaining animals were retained for the recovery phase.

2. MATERIALS AND METHODS

Ophthalmic examinations were performed on animals (Groups 1-3) prior to the initiation of dosing (PID) on PID Day 7 for males and PID Day 8 for females, except for Animal 88 examined on PID Day 9, and on Day 15 for males and Day 16 for females. Tropicamide 1% was administered topically to each eye to facilitate the examination. Indirect ophthalmoscopy was used for examinations. Handheld slit lamp biomicroscopy was also used at the discretion of the examiner.

3. DATA ACQUISITION

Pristima Preclinical Data Management Suite (Version 7.4.3) was used to record ophthalmology data.

4. DATA MANAGEMENT AND ARCHIVES

All raw data and the original report pertaining to this phase of the study are retained at Pfizer, DSRD, Groton, CT (USA). A copy of this report is appended to the study report.

Materials including raw data and documents electronically archived are retained in the Pfizer OpenLab archive system or locked and retained in the source computerized system, as defined per SOP.

5. RESULTS

An incidence summary of ophthalmic findings is presented in Table 2. Individual animal ophthalmic findings are included in Appendix 3.

Ophthalmic examinations of rats performed prior to initiation of dosing were within normal limits, except for incidental findings noted in the following animals: mild unilateral vitreous hemorrhage in Animal 8, minimal unilateral tortuous retinal vessels in Animal 10, minimal unilateral vitreous hyaloid remnant in Animal 11, and mild unilateral keratic precipitates in Animal 14 and Animal 41.

No test article-related ophthalmic findings were observed in rats at the end of the dosing phase.

The mild unilateral keratic precipitates observed on Day 16 in Animal 49 is a recognized spontaneous finding in Wistar Han rats and was not considered test article related (Williams, 2013). The Day 15 ophthalmic findings in Animals 8, 10, 11, 14, and 41 were consistent with those observed on PID Day 7.

Ophthalmic examinations were not conducted during the recovery phase due to the lack of test article-related changes at the end of the dosing phase.

6. INTEGRATED SUMMARY AND DISCUSSION OF RESULTS

Clinical ophthalmic parameters of rats examined in this study were not affected following 3 intramuscular doses of BNT162b2 (V9) or BNT162b3c administered 1 week apart.

7. REFERENCES

Williams, DL. Laboratory animal ophthalmology. In: Gelatt KN, Gilger BC, Kern TJ, eds. Veterinary Ophthalmology. 5th ed. Vol 2. Ames, IA: Wiley-Blackwell; 2013:1698.

Document Approval Record

Document Name:	DSRD Ophthalmology Report
Document Title:	20GR142: DSRD Ophthalmology Report

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	04-Nov-2020 18:45:48	Author Approval

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PHASE REPORT

17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

20GR142 (WORK ORDER 4)

SERVICE PERFORMED BY:
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Version summary:

VERSION	ISSUE DATE	CHANGE
0.1	24 Sept 2020	Draft report
0.2	05 Oct 2020	Sponsor review and data table added in Appendix
0.3	07 Oct 2020	Typo edits, margins and data table adaptation
1.0	29 Oct 2020	Final report

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1. PURPOSE

This Phase Report describes the activities completed by VisMederi applying the Microneutralization (MN) assay for serological detection of SARS-CoV-2 specific neutralizing antibodies in animal sera relative to the "Work order 4" agreed between VisMederi Srl and Pfizer.

2. STUDY MANAGEMENT

The BNT162b2 (V9) and BNT162b3c candidate COVID-19 vaccines, based on an RNA platform and target the SARS-CoV-2 spike protein, were evaluated through the 20GR142 study for toxicity and immune response development.

BNT162b2 (V9) and BNT162b3c were administered by intramuscular (IM) doses once weekly for a total of 3 doses to male and female Wistar Han (Crl:WI[Han]) rats. Animals received the vehicle or test article at doses of 30 µg RNA/Dose Day followed by a 3-week recovery phase. Group designations and doses are indicated in the table below.

Experimental Design				
Group Number	**Test Article or Vehicle Dose/Dose Day (µg/Dose Day)**	**Dose Volume (µL/injection site)[a]**	**Animal Numbers**	
			Males	**Females**
1	0[b]	60	1-15	46-60
2	30[c]	60	16-30	61-75
3	30[d]	60	31-45	76-90

a. Each animal received a single injection on each dose day.
b. Sterile saline
c. BNT162b2(V9)
d. BNT162b3c

Doses were administered by a single intramuscular injection on each dosing day (60 µL) administered into the left hindlimb quadriceps muscle on Days 1, 8, and 15.

The first 10 animals/sex/group, by ascending animal order, were designated for necropsy at the end of the dosing phase. The remaining animals were retained for the recovery phase.

Samples for antibody response to the vaccine components were collected prior to dose initiation (PID) on PID Day 8 (Day -5), during the dosing phase on Day 17, and during the recovery phase (RP) on RP Day 21 (Day 38).

VisMederi performed immunogenicity tests on rats samples, testing for detection of neutralizing antibody titers to wild type live Sars-CoV-2 virus.
The assay was performed according to VisMederi internal working instruction "Microneutralization CPE-based assay for SARS-COV-2" (WI-MNSARS-CoV-2), in accordance with the Good Clinical Laboratory Practice 2009/2013 (GCLP).

VisMederi received, on August 24th 2020, 210 rat's serum samples, from Pfizer DSRD – Eastern Point Road, Groton, CT 06340, USA, for study 20GR142 with BNT162b2 (V9) and BNT162b3c. In particular, the shipment contained prior to initiation of dosing (PID) Day 8 (Day -5) and dosing phase Day 17 time-points samples of 90 animals and recovery phase (RP) Day 21 (Day 38) time-points from 30 rats.

Upon arrival, all samples passed a visual check of the physical characteristics and correspondence with material shipping inventory, according to the SOP-HBM of VisMederi, and they were stored in a freezer at -20°C (VM-F-009).

Each serum sample of study 20GR142 has been tested in duplicate for serological detection of SARS-CoV-2 specific neutralizing antibodies.
The SARS-CoV-2 2019 live wild type virus 2019-nCoV strain 2019-nCov/Italy-INMI1 was obtained by VisMederi Srl from the European Virus Archive Global (EVAg).
The strain information are available at the following link:
https://www.european-virus-archive.com/virus/human-2019-ncov-strain-2019-ncovitaly-inmi1

The virus growth was carried out by VisMederi Research Srl, according to VisMederi Research procedure "Virus Growth in cell culture" (SOP-VGC) in epithelial cell line, VERO E6 cells (from kidney of a normal monkey Cercopithecus aethiops) provided by the American Type Culture Collection (ATCC - CRL 1586).

The internal virus batch applied for MN analyses was VMR_SARSCOV2VEROE6_280420_C1.
The Microneutralization assay for SARS-CoV-2 on rat sera samples were performed on 8th – 11th September 2020 in the VisMederi BSL3 laboratories in accordance to the SOP-HSAL of VisMederi.

QA Statement and Regulatory Statement

The work was conducted in accordance with the procedures in force and following the GCLP guidelines and under ISO 9001:2015.
All the laboratory staff involved was trained in recording the raw data of the study in a timely and accurate manner, and aware of the responsibility of the quality of the data produced.
Independent laboratory audits are conducted periodically to ensure the quality of work and data integrity.
Equipment used are periodically maintained, calibrated and qualified as appropriate.

All the documentation related to the study is archived in a secure place in compliance with the ISO 27001 (both in electronic and paper format).
No significant laboratory events or deviations have occurred during the study that could have impacted the generated results.

3. TEST PROCEDURE

The MN-CPE (Microneutralization based on Cytopathic effect) method is a specific technique used for the identification of virus-specific neutralizing antibodies against live viruses which are able to prevent the virus infection. This assay is a fundamental test in virology, immunology, vaccine assessment and epidemiology studies.

The assay was performed following the VisMederi procedure "WI-MNSARS-CoV-2", and the main phases are described as follows:

- Virus Titration
- Back titration
- Microneutralization

Virus titration

The virus, ten-fold serially diluted in suitable MN medium, was transferred to a plate containing confluent VERO E6 cell monolayers.
After incubation of 3 days the plate was observed under an inverted microscope and the wells were scored as positive/negative for Cytopathic effect (CPE).
The titer was calculated using the Reed-Muench method, obtaining $10^{7.59}$ TCID50/mL as result. The stock virus was then applied in the MN assay at a proper dilution in order to contain 2000TCID50/mL in the working virus solution.

MN assay

Serum samples were heat inactivated for 30 minutes at 56°C, then two-fold serially diluted starting from 1:10 up to 1:5120 and were mixed with an equal volume of viral solution.

Duplicate runs for each sample were performed in two different plates.

The serum-virus mixture was incubated for 1 hour at 37°C, in a humidified atmosphere with 5% CO_2. After the incubation time, 100 µl of the mixture for each dilution was added in duplicate to a cell plate containing a healthy and sub confluent-to confluent VERO E6 cell lawn and incubated for 3 days in the CO_2 incubator at 37°C and 5% CO_2. The readout was achieved through inverted optical microscopy in order to discriminate wells as positive/negative for Cytopathic effect (CPE).

The Microneutralization titer (MNt) of each titrated sample corresponded to the reciprocal of the highest sample dilution able to protect from CPE at least 50% of the cell monolayer. If no neutralization was observed (MNt <10) an arbitrary value of 5 was reported.

Back titration and reference samples

To verify the virus workload in the solution applied in the assay, the virus working solution was titrated in each MN session. The back titrations performed in both sessions for this study confirmed virus titers within the defined acceptance range of (b) (4)

In addition, each test session included runs of specific reference sera: a positive and a negative serum.

The positive control (PCS) used in every test run, is a human plasma sample collected from a COVID-19 convalescent patient. The sample code TLS-8 was previously tested

by MN and by ELISA for SARS-CoV-2 antibody titer, providing high positive response confirmed by multiple repetitions.
The negative control sample (NCS) used was a human serum depleted of IgA, IgM and IgG, provided by Sigma Aldrich, cod. S5393 batch 108M4791V.

4. ACCEPTANCE CRITERIA

In agreement with WI-MNSARS-CoV-2, the following internal quality controls have been satisfied in each session of analysis for the Study 20GR142 samples, therefore results were considered reliable and acceptable.

Virus titer evaluation:

- The back titration of the working viral solution lies within the defined target range of (b) (4)

MN results acceptability of each MN plate:

- The cell control (CC) showed a healthy cell monolayer and no evidence of CPE
- The virus control (VC) wells showed cytopathic effect.

MN results acceptability of each sample:

- The duplicate neutralization titers of each serum sample were within a range of ±(b) (4) .

MN results acceptability of each analysis session:

- the positive control sample (PCS) showed a positive titer, in agreement with previous data,
- the negative control sample (NCS) with absent antibody titer showed a negative response.

Since all the acceptability criteria were met, no retest was necessary.

5. DATA RELEASE

Test results were recorded through dedicated forms, attachments of the VisMederi WI "WI-MNSARS-CoV-2", and transferred in an excel data entry sheet: PFZ_20GR142-WO4_MN-SarsCov2_V2_20200924_GL.xlsx

This report shows the full set of data in Appendix 1 and 2 tables.

Data entry description

The data tables present three sections:

- Sample identification
- Raw Data
- Derived values as geometric mean of duplicate tests

Each subject is identified in a row of table by Sample ID, gender and administered dose of vaccine. Any Study day is showed in following columns as duplicate results "T1A" and "T1B", each one is used for a replicate titer of the same sample. Last columns shows the geometric mean calculated from the two replicate titers for each study visit.

The following table shows geometric mean titers for grouped subjects by sex and for vaccine administered.

Table 1: Overview of GMTs for each dose group, by sampling day and sex

Study Day	Sex	Saline	30µg BNT162b2(V9)	30µg BNT162b3c
PID Day 8 (Day -5)	Male	5	5	5
	Female	5	5	5
Day 17	Male	5	1114	993
	Female	5	2501	1810
RP Day 21 (Day 38)	Male	5	5120	3880
	Female	5	5120	3880
PID = prior to dose initiation; RP = Recovery Phase				

Administration of 3 once weekly doses of BNT162b2 (V9) or BNT162b3c elicited SARS-CoV-2 neutralizing antibody responses in males and females at the end of the dosing (Day 17) and recovery phases (Day 21) of the study. SARS-CoV-2 neutralizing antibody responses were not observed in animals prior to vaccine administration or in saline-administered control animals.

6. REFERENCES

- Manenti A, Maggetti M, Casa E, et al (2020). Evaluation of SARS-CoV-2 neutralizing antibodies using of a CPE-based Colorimetric live virus micro-neutralization assay in human serum samples. Journal of Medical Virology. doi: 10.1002/jmv.25986.
- Reed, L.J.; Muench, H. (1938). "A simple method of estimating fifty percent endpoints". The American Journal of Hygiene. 27: 493–497.
- Algaissi A, Hashem AM. (2020). Evaluation of MERS-CoV Neutralizing Antibodies in Sera Using Live Virus Microneutralization Assay. Methods in molecular biology (Clifton, N.J.) vol. 2099: 107-116.
- Good Clinical Laboratory Practice GCLP – 2009/2013
- OECD Principles on Good Laboratory Practice (ENV/MC/CHEM(98)17)
- UNI EN ISO 9001:2015
- UNI EN ISO 27001:2017
- "WI-MNSARS-CoV-2" Working Instruction "Microneutralization CPE-based assay for Sars-Cov-2"
- "HSAL" Handling and safety for activities in BSL2 and BSL3 Laboratories – VisMederi procedure
- "HBM" Handling Of Biological Material – Vismederi procedure
- "HCC" Handling Cell Cultures – VisMederi Research procedure
- "MRR" Management and Release of Results – VisMederi procedure
- "VGC" Virus Growth in Cell culture – VisMederi Research procedure

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7. APPENDICES

Appendix 1: data table for Male

Sample ID	Gender	Dose	Dose Units	T1A-DAY8	T1A-DAY17	T1A-DAY21	T1B-DAY8	T1B-DAY17	T1B-DAY21	Geometric Mean Day8	Geometric Mean Day17	Geometric Mean Day21
001M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
002M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
003M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
004M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
005M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
006M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
007M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
008M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
009M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
010M	Male	0	µg/kg	5	5		5	5		5.0	5.0	
011M	Male	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
012M	Male	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
013M	Male	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
014M	Male	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
015M	Male	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
016M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
017M	Male	30	µg/kg	5	640		5	640		5.0	640.0	
018M	Male	30	µg/kg	5	2560		5	1280		5.0	1810.2	
019M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
020M	Male	30	µg/kg	5	320		5	640		5.0	452.5	
021M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
022M	Male	30	µg/kg	5	640		5	1280		5.0	905.1	
023M	Male	30	µg/kg	5	1280		5	1280		5.0	1280.0	
024M	Male	30	µg/kg	5	1280		5	1280		5.0	1280.0	
025M	Male	30	µg/kg	5	1280		5	1280		5.0	1280.0	
026M	Male	30	µg/kg	5	320	5120	5	320	5120	5.0	320.0	5120.0
027M	Male	30	µg/kg	5	640	5120	5	320	5120	5.0	452.5	5120.0
028M	Male	30	µg/kg	5	1280	5120	5	1280	5120	5.0	1280.0	5120.0
029M	Male	30	µg/kg	5	320	5120	5	640	5120	5.0	452.5	5120.0
030M	Male	30	µg/kg	5	2560	5120	5	5120	5120	5.0	3620.4	5120.0
031M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
032M	Male	30	µg/kg	5	320		5	320		5.0	320.0	
033M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
034M	Male	30	µg/kg	5	1280		5	2560		5.0	1810.2	
035M	Male	30	µg/kg	5	160		5	160		5.0	160.0	
036M	Male	30	µg/kg	5	640		5	640		5.0	640.0	
037M	Male	30	µg/kg	5	160		5	320		5.0	226.3	
038M	Male	30	µg/kg	5	1280		5	1280		5.0	1280.0	
039M	Male	30	µg/kg	5	320		5	320		5.0	320.0	
040M	Male	30	µg/kg	5	2560		5	2560		5.0	2560.0	
041M	Male	30	µg/kg	5	2560	5120	5	5120	5120	5.0	3620.4	5120.0
042M	Male	30	µg/kg	5	1280	5120	5	1280	5120	5.0	1280.0	5120.0
043M	Male	30	µg/kg	5	2560	5120	5	2560	5120	5.0	2560.0	5120.0
044M	Male	30	µg/kg	5	640	2560	5	640	2560	5.0	640.0	2560.0
045M	Male	30	µg/kg	5	1280	2560	5	1280	2560	5.0	1280.0	2560.0

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Appendix 2: data table for Female

Sample ID	Gender	Dose	Dose Units	T1A-DAY8	T1A-DAY17	T1A-DAY21	T1B-DAY8	T1B-DAY17	T1B-DAY21	Geometric Mean Day8	Geometric Mean Day17	Geometric Mean Day21
046F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
047F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
048F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
049F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
050F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
051F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
052F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
053F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
054F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
055F	Female	0	µg/kg	5	5		5	5		5.0	5.0	
056F	Female	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
057F	Female	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
058F	Female	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
059F	Female	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
060F	Female	0	µg/kg	5	5	5	5	5	5	5.0	5.0	5.0
061F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
062F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
063F	Female	30	µg/kg	5	640		5	640		5.0	640.0	
064F	Female	30	µg/kg	5	2560		5	2560		5.0	2560.0	
065F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
066F	Female	30	µg/kg	5	2560		5	2560		5.0	2560.0	
067F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
068F	Female	30	µg/kg	5	2560		5	2560		5.0	2560.0	
069F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
070F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
071F	Female	30	µg/kg	5	2560	5120	5	2560	5120	5.0	2560.0	5120.0
072F	Female	30	µg/kg	5	2560	5120	5	5120	5120	5.0	3620.4	5120.0
073F	Female	30	µg/kg	5	5120	5120	5	5120	5120	5.0	5120.0	5120.0
074F	Female	30	µg/kg	5	1280	5120	5	1280	5120	5.0	1280.0	5120.0
075F	Female	30	µg/kg	5	1280	5120	5	1280	5120	5.0	1280.0	5120.0
076F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
077F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
078F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
079F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
080F	Female	30	µg/kg	5	640		5	640		5.0	640.0	
081F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
082F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
083F	Female	30	µg/kg	5	1280		5	1280		5.0	1280.0	
084F	Female	30	µg/kg	5	5120		5	5120		5.0	5120.0	
085F	Female	30	µg/kg	5	2560		5	2560		5.0	2560.0	
086F	Female	30	µg/kg	5	320	1280	5	320	1280	5.0	320.0	1280.0
087F	Female	30	µg/kg	5	5120	5120	5	5120	5120	5.0	5120.0	5120,0
088F	Female	30	µg/kg	5	1280	5120	5	2560	5120	5.0	1810.2	5120.0
089F	Female	30	µg/kg	5	640	5120	5	640	5120	5.0	640.0	5120.0
090F	Female	30	µg/kg	5	5120	5120	5	5120	5120	5.0	5120.0	5120.0

C O N F I D E N T I A L

CLINICAL PATHOLOGY REPORT: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Testing Facility Study Number: 20GR142

Alternative Test Article Identifier(s):

PF-07302048: Generic number for COVID-19 vaccine program

BNT162b2 (V9): BNT162b2 (Version 9); RBP020.2; PF-07305885

BNT162b3c: BNT162b3; RBP020.8; PF-07315256

TESTING FACILITY
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TEST SITE
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SIGNATURES

I was responsible for the principal investigator activities conducted in support of this study and confirm that this report accurately reflects my interpretation of the clinical pathology data and that my portions of the study were conducted in compliance with GLP regulations with the exceptions noted; (see GLP Compliance Statement).

(b) (6)

Clinical Pathologist
Principal Investigator

Quality Assurance Statement Signature

The signature for the following individual applies only to the Pearl River, NY Quality Assurance Statement contained in this study report.

(b) (6) Pfizer Inc, Pearl River, NY.

For signatures see the Document Approval Record located on the last page of this report.

GLP COMPLIANCE STATEMENT

This portion of the study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58) with the exception of the analyses of alpha-1 acid glycoprotein (A1AGP) and alpha-2-macroglobulin (A2M) which were conducted under non-GLP conditions, but according to fit-for-purpose methods. This exception did not have an impact on the integrity or data interpretation of the study.

TABLE OF CONTENTS

1. INTRODUCTION

BNT162b2 (Version 9 [V9]) and BNT162b3c are candidate COVID-19 vaccines, which consist of an LNP-encapsulated RNA encoding the SARS-CoV-2 spike protein or its derivatives, were administered by intramuscular (IM) doses once weekly for a total of 3 doses to male and female Wistar Han (Crl:WI[Han]) rats. Animals received the vehicle or test article at doses of 0 or 30 µg RNA/Dose Day followed by a 3-week recovery phase. Group designations and doses are indicated in the table below.

Experimental Design				
Group Number	Test Article or Vehicle Dose (µg RNA/Dose Day)	Dose Volume (µL/injection site)[a]	Animal Numbers	
			Males	Females
1	0[b]	60	1-15	46-60
2	30[c]	60	16-30	61-75
3	30[d]	60	31-45	76-90

a. Each animal received a single injection on each dose day.
b. Sterile saline.
c. BNT162b2 (V9).
d. BNT162b3c.

Doses were administered by a single intramuscular injection on each dosing day (60 µL) administered into the left hindlimb quadriceps muscle on Days 1, 8, and 15.

The first 10 animals/sex/group, by ascending animal order, were designated for necropsy at the end of the dosing phase. The remaining animals were retained for the recovery phase.

2. MATERIALS AND METHODS

Clinical laboratory measurements were completed at the Testing Facility (Pfizer DSRD, Groton, CT) and clinical laboratory measurements interpretation was completed at the Test Site (Pfizer DSRD, Pearl River, NY).

2.1. Clinical Laboratory Measurements

Clinical laboratory parameters were evaluated in samples collected from all animals as listed in each section below.

Bone marrow smears were prepared for all animals. Bone marrow smear slides were stained with May-Grunwald Giemsa and were not examined.

Schedule for Collection of Samples for Clinical Laboratory Measurements			
Parameter	**Day of Study**		
	Dosing Phase		**Recovery Phase**
	Day 4	Day 17[e]	Day 22
Hematology	X[a,c]	X[c]	X[c]
Coagulation	NA	X[c]	X[c]
Clinical Chemistry (Core Chemistry)	X[b,c]	X[c]	X[c]
Clinical Chemistry (Other Biomarkers – Acute Phase Proteins)/Serum[d]	X[b,c]	X[c]	X[c]
Urinalysis	NA	X	X

NA = Not applicable; X = Scheduled Collection.
a. First 7 animals/sex/group.
b. Last 8 animals/sex/group.
c. Blood samples were collected from animals in a fasted state, with the exception of same day redraws.
d. Assay performed using shared clinical chemistry sample.
e. Evaluated on animals scheduled for necropsy.

Blood Collection			
Parameter	**Collection Condition**	**Approximate Blood Volume**	**Anticoagulant**
Clinical Chemistry	Nonterminal[a, b]	0.7 mL	None-Serum Separator Tube
Clinical Chemistry	Terminal	2.5 mL	None-Serum Separator Tube
Hematology	Nonterminal[a,b]	0.5 mL	K_2EDTA
Hematology	Terminal	2.0 mL	K_2EDTA
Coagulation	Terminal	2.0 mL	3.2% sodium citrate
Biomarkers (Clinical Chemistry)	Nonterminal[a,b]	using shared sample	None-Serum Separator Tube
Biomarkers (Clinical Chemistry)	Terminal	Using shared sample	None-Serum Separator Tube

a. Blood samples were collected under anesthesia.
b. This volume was based on collecting blood for one parameter per group of animals and the other parameter on another group of animals.

2.1.1. Hematology and Coagulation

Blood samples were analyzed for:

Red Blood Cells (RBC)	Red Cell Distribution Width (RDW)
Hemoglobin (HGB)	Reticulocytes (RETIC)
Hematocrit (HCT)	Platelets (PLT)
Mean Cell Volume (MCV)	Mean Platelet Volume (MPV)
Mean Cell Hemoglobin (MCH)	White Blood Cells (WBC)
Mean Cell Hemoglobin Concentration (MCHC)	White Cell Differential

Blood smears were prepared for the first 7 animals on Day 4 and all animals on Dosing Phase Day 17 and Recovery Phase Day 21 (Groups 1-3).
Blood cell morphology was evaluated microscopically on 5 animals of each sex from all groups at both scheduled necropsies (ie, at dosing and recovery phases).

Blood samples were analyzed for:

Activated Partial Thromboplastin Time (APTT)	Prothrombin Time (PT_Rat)
Fibrinogen (FIB)	

2.1.2. Clinical Chemistry

Core Chemistry

Serum samples were analyzed for:

Alanine Aminotransferase (ALT)	Globulin (GLOB)
Aspartate Aminotransferase (AST)	Albumin/Globulin Ratio (AG)
Alkaline Phosphatase (ALP)	Blood Urea Nitrogen (BUN)
Gamma Glutamyltransferase (GGT)	Creatinine (CREA)
Bilirubin, Total (TBIL)	Phosphorus (PHOS)
Cholesterol (CHOL)	Calcium (CA)
Triglyceride (TRIG)	Sodium (NA)
Glucose (GLUC)	Potassium (K)
Total Protein (TP)	Chloride (CL)
Albumin (ALB)	

Serum indices for hemolysis, icterus, and lipemia were performed.

Other Biomarkers

Serum Biomarker Sample Collection

Serum samples were analyzed for:

alpha-1-acid glycoprotein (A1AGP)	alpha-2-macroglobulin (A2M)

2.1.3. Urinalysis

Urine samples were collected overnight at scheduled necropsy. Urine samples were analyzed for:

Color	Protein (PRO)
Clarity	Blood
pH	Bilirubin (BIL)
Glucose Urine (GLU)	Specific Gravity (SG)
Ketones (KET)	Volume

Microscopic examination of sediment for formed elements was performed on 5 animals of each sex from all dose groups at both scheduled necropsies (ie, dosing and recovery phases).

2.2. Statistical Analysis

Statistical analyses of hematology, coagulation, clinical chemistry, and urinalysis data was conducted in Pristima. All analyses were performed separately for each sex.

Descriptive statistics were generated for each parameter and group at each scheduled sampling time or each time interval. Statistical tests were conducted at the 5% and 1% significance levels.

Analyses of hematology, coagulation, clinical chemistry, and urinalysis parameters were done on measurements collected for each animal at the scheduled sampling times or time intervals.

A nonparametric (rank-transform) one way analysis of variance (ANOVA) on all groups was conducted, with two-sided pairwise comparisons of Groups 2 and 3 to Group 1 using Dunnett's test. Average ranks were assigned to ties.

3. DATA ACQUISITION

Cerner HNA Millennium Laboratory Information System (Version 2018.01) was used to record clinical laboratory measurement data.

4. DATA MANAGEMENT AND ARCHIVES

Raw data, documentation, protocol and amendments, final report, and any specimens generated at the Test Facility as the result of the study are retained at Pfizer, Groton, CT.

Materials including raw data and documents electronically archived are retained in the Pfizer OpenLab archive system or locked and retained in the source computerized system, as defined per SOP.

Materials are retained in accordance with the Enterprise Records Retention Schedule.

5. RESULTS

5.1. Clinical Laboratory Measurements

5.1.1. Hematology and Coagulation

Group mean hematology and coagulation data are presented in Table 6. Individual animal hematology and coagulation data are included in Appendix 7. Results indicated below are test article group mean values compared with control group mean values.

Dosing Phase

Test article-related hematology and coagulation findings were similar in rats administered either BNT162b2 (V9) or BNT162b3c and included higher mean white blood cell (WBC) counts and fibrinogen concentrations, lower (Day 4) and higher (Day 17) reticulocyte counts, and lower red blood cell mass (red blood cell [RBC] count, hemoglobin [HGB] and hematocrit [HCT], as represented by HCT in Text Table 1) compared with controls.

Higher WBC primarily involved neutrophils, monocytes and large unstained cells (LUC) but also affected eosinophils and basophils. They were present on Days 4 and 17, with higher counts on Day 17 than Day 4. On Day 17, there were also test article-related higher fibrinogen concentrations in both sexes. Hypersegmented neutrophils were present on peripheral blood smears of test article-treated animals.

In addition, there were test article-related transiently lower reticulocyte counts on Day 4, and higher reticulocytes on Day 17 (females only) with attendant expected changes in RBC indices (higher mean cell hemoglobin concentration [MCHC; males] on Day 4; lower mean cell hemoglobin [MCH] and higher red cell distribution width [RDW] on Day 17; both sexes). These were associated with lower RBC mass on Days 4 & 17 (comparable on both days or slightly lower on Day 17).

Text Table 1. Test Article-Related Hematology and Coagulation Parameter Effects (Mean Control Values and Ratio Relative to Control Mean)

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	30	0	30	30
HCT (%)						
4D	48.04	0.90x	0.91x	44.91	0.93x	0.93x
17D	42.61	0.90x	0.92x	41.67	0.91x	0.89x
MCH (pg)						
4D	18.51	-	-	18.37	-	-
17D	18.27	0.96x	-	18.62	0.97x	0.96x
MCHC (g/dL)						
4D	31.24	1.04x	1.03x	32.34	-	-
17D	32.46	-	-	33.18	-	-
RDW (%)						
4D	12.27	-	-	11.11	-	-
17D	11.63	1.21x	1.18x	11.33	1.18x	1.18x
RETIC (10e3/uL)						
4D	392.1	0.27x	0.27x	301.7	0.43x	0.44x
17D	178.8	-	-	168.9	1.31x	1.20x
WBC (10e3/uL)						
4D	7.60	1.41x	1.28x	6.01	1.30x	1.43x
17D	3.84	2.30x	2.24x	2.16	2.64x	2.95x
NEUT (10e3/uL)						
4D	1.083	2.28x	2.00x	0.920	2.51x	3.13x
17D	0.674	6.60x	6.46x	0.409	6.04x	7.04x
MONO (10e3/uL)						
4D	0.109	1.83x	1.96x	0.093	1.89x	2.52x
17D	0.071	3.30x	3.58x	0.056	2.75x	3.14x
EO (10e3/uL)						
4D	0.081	-	-	0.057	-	2.16x
17D	0.056	2.52x	2.18x	0.029	3.17x	3.34x
BASO (10e3/uL)						
4D	0.016	1.88x	2.31x	0.009	1.89x	2.67x
17D	0.003	5.67x	6.33x	0.001	8.00x	10.00x
LUC (10e3/uL)						
4D	0.046	4.07x	3.98x	0.030	4.20x	4.43x
17D	0.026	8.04x	12.42x	0.010	13.20x	19.00x

Text Table 1. Test Article-Related Hematology and Coagulation Parameter Effects (Mean Control Values and Ratio Relative to Control Mean) - Continued

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	30	0	30	30
FIB (mg/dL)						
17D	253.1	2.36x	2.39x	217.2	2.49x	2.59x

Control mean values and the ratio of the test article-related findings relative to control means are listed.
- = Not test article related; BASO = Basophil, absolute; D = Day; EO = Eosinophil, absolute; FIB = Fibrinogen; HCT = Hematocrit; LUC = Large unstained cells, absolute; MCH = Mean cell hemoglobin; MCHC = Mean cell hemoglobin concentration; MONO = Monocyte, absolute; NEUT = Neutrophil, absolute; RDW = Red cell distribution width; RETIC = Reticulocyte, absolute; WBC = White blood cells.

Recovery Phase

All test article related changes were fully reversed after a 3-week recovery period, with the exception of higher RDW (Text Table 2).

Text Table 2. Test Article-Related Hematology and Coagulation Parameter Effects (Mean Control Values and Ratio Relative to Control Mean)

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	30	0	30	30
RDW (%)						
R22	11.93	1.13x	1.12x	10.80	1.21x	1.23x

Control mean values and the ratio of the test article-related findings relative to control means are listed.
R = Recovery Day; RDW = Red cell distribution width.

Other statistically significant or apparent differences between test article and control group hematology and coagulation parameters were not test article related due to one or more of the following: small magnitude of the difference, inconsistent direction of the difference, and general overlap in magnitude of individual values with controls.

5.1.2. Clinical Chemistry

Group mean clinical chemistry data are presented in Table 7. Individual animal clinical chemistry data are included in Appendix 8. Results indicated below are test article group mean values compared with control group mean values.

Dosing Phase

Test article-related clinical chemistry findings were similar in rats administered 30 µg RNA/dosing day of either BNT162b2 (V9) or BNT162b3c and included higher mean alpha-1 acid glycoprotein (A1AGP) and alpha-2-macroglobulin (A2M) and lower albumin:globulin (AG) ratios (primarily due to lower albumin with slight contribution from higher globulins) on Days 4 and 17 in both sexes compared with controls (Text Table 3).

Text Table 3. Test Article-Related Clinical Chemistry Parameter Effects (Mean Control Values and Ratio Relative to Control Mean)

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	30	0	30	30
ALB (g/dL)						
4D	3.98	0.93x	0.92x	4.16	0.86x	0.90x
17D	3.50	-	-	3.60	0.85x	0.86x
GLOB (g/dL)						
4D	2.13	-	-	2.10	-	1.05x
17D	1.89	1.10x	1.07x	1.84	1.04x	-
AG						
4D	1.88	0.90x	0.90x	1.98	0.86x	0.85x
17D	1.85	0.89x	0.89x	1.96	0.82x	0.85x
A1AGP						
4D	174.358	9.42x	13.49x	239.774	7.95x	6.99x
17D	47.672	38.51x	42.40x	95.959	15.55x	17.21x
A2M						
4D	113.4	20.44x	34.99x	212.1	3.32x	4.18x
17D	14.0	70.76x	128.16x	33.1	15.74x	17.89x

Control mean values and the ratio of the test article-related findings relative to control means are listed.
- = Not test article related; A1AGP = alpha-1 acid glycoprotein; A2M = alpha-2-macroglobulin; AG = Albumin/globulin ratio; ALB = Albumin; D = Day; GLOB = Globulin; TP = Protein, total.

Recovery Phase

All test article related changes were fully reversed after a 22-day recovery period, with the exception of higher globulins in males administered BNT162b2 (V9) and females administered BNT162b2 (V9) and BNT162b3c, and lower AG ratio in females administered BNT162b2 (V9) (see Text Table 4).

Text Table 4. Test Article-Related Clinical Chemistry Parameter Effects (Mean Control Values and Ratio Relative to Control Mean)

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	30	0	30	30
GLOB (g/dL)						
R22	2.10	1.08x	-	2.26	1.06x	1.07x
AG						
R22	1.76	-	-	1.90	0.91x	-

Control mean values and the ratio of the test article-related findings relative to control means are listed.
- = Not test article related; AG = Albumin/globulin ratio; GLOB = Globulin; R = Recovery Day.

Other statistically significant or apparent differences between test article and control group clinical chemistry parameters were not test article related due small magnitude of the difference and general overlap in magnitude of individual values with controls.

5.1.3. Urinalysis

Group mean urinalysis data are presented in Table 8. Individual animal urinalysis data are included in Appendix 9.

Dosing and Recovery Phases

There were no test article-related findings on urinalysis.

All statistically significant or apparent differences in urinalysis parameters between test article and control group were not test article related due small magnitude of the difference and general overlap in magnitude of individual values with controls.

6. INTEGRATED SUMMARY AND DISCUSSION OF RESULTS

Male and female Wistar-Han rats were administered either BNT162b2 (V9) or BNT162b3c at 30 µg RNA/Dose Day by intramuscular (IM) injection once weekly for 3 weeks, resulting in nonadverse findings in hematology and clinical chemistry parameters compared with control animals.

All clinical pathology findings (type and magnitude) were generally comparable 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 males and females on Days 4 and/or 17 and included higher acute phase proteins (alpha-1

acid glycoprotein [A1AGP; 7.0x – 42x controls], alpha-2-macroglobulin [A2M; 3.3x – 128x] and fibrinogen [2.4x-2.6x]), lower albumin:globulin (AG; 0.90x - 0.82x; primarily due to lower albumin [0.93x – 0.85x] with slight contribution from globulins [1.04x – 1.10x]), and higher WBC (1.28x - 2.95x; primarily involving neutrophils, monocytes and LUC, which typically represent large mononuclear cells). Hypersegmented neutrophils present on peripheral blood smears were considered 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). Collectively, these findings were consistent with expected immune responses to vaccines. Microscopic correlates included 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 centers of lymphoid organs.

In addition, there were transiently lower reticulocyte counts on Day 4 (0.44x - 0.27x), and higher reticulocytes on Day 17 (1.20x - 1.31x; females only), with minor lower red cell mass on Days 4 and 17 (HCT; 0.93x - 0.89x). Lower reticulocytes were interpreted to be a transient effect of innate immune responses (Abreu et al, 2018; Brooks et al, 2017; Kim et al, 2014; Wrighting & Andrews, 2006).

All test article related changes were fully reversed after a 3-week recovery period, with the exception of higher RDW in males and females administered BNT162b2 (V9) (1.13x and 1.21x, respectively) and BNT162b3c (1.12x and 1.23x, respectively), higher globulins in males administered BNT162b2 (V9) (1.08x) and females administered BNT162b2 (V9) (1.06x) and BNT162b3c (1.07x), and lower AG ratio in females administered BNT162b2 (V9) (0.91x).

In conclusion, clinical pathology findings in rats administered BNT162b2 (V9) or BNT162b3c were consistent with expected immune responses to vaccines or secondary to inflammation and included higher acute phase proteins (A1AGP, A2M and fibrinogen), higher WBC (primarily neutrophils, monocytes and LUC), lower red blood cell mass and transiently lower reticulocytes.

7. REFERENCES

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Medical Quality Assurance

Quality Assurance Statement

Title: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study: 20GR142

In accordance with Pfizer policies and Medical Quality Assurance procedures for Good Laboratory Practice (GLP), the conduct of this portion of this study has been inspected and/or audited as follows.

Phase Inspected	Audit/Inspection Date GMT	Reporting Date GMT
Test Site Protocol Review	24-Jun-2020 to 25-Jun-2020	25-Jun-2020
Protocol Amendment #1	02-Jul-2020 to 02-Jul-2020	02-Jul-2020
Protocol Amendment #4	04-Sep-2020 to 04-Sep-2020	04-Sep-2020
Report: Clinical Pathology	20-Oct-2020 to 30-Oct-2020	03-Nov-2020
Report: Pathology	20-Oct-2020 to 30-Oct-2020	03-Nov-2020

In addition Routine Facility and Process audits are conducted in accordance with MQA SOPs and Site Monitoring Plans.

(b) (6)

Document Approval Record

Document Name:	Clinical Pathology Report
Document Title:	20GR142 Clinical Pathology Report

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	09-Nov-2020 21:24:21	Quality Assurance Approval
	10-Nov-2020 20:16:43	Author Approval

C O N F I D E N T I A L

ANATOMIC PATHOLOGY REPORT: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Testing Facility Study Number: 20GR142

Alternative Test Article Identifier(s):

PF-07302048: Generic number for COVID-19 vaccine program

BNT162b2 (V9): BNT162b2 (Version 9); RBP020.2; PF-07305885

BNT162b3c: BNT162b3; RBP020.8; PF-07315256

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Anatomic Pathology Report for Study 20GR142

SIGNATURES

I was responsible for the principal investigator activities conducted in support of this study and confirm that this report accurately reflects my interpretation of the organ weight, macroscopic, and microscopic data and that my portions of the study were conducted in compliance with GLP regulations (see GLP Compliance Statement).

(b) (6)

Quality Assurance Statement Signature

The signature for the following individual applies only to the Pearl River, NY Quality Assurance Statement contained in this study report.

(b) (6) Pfizer Inc, Pearl River, NY.

For signatures see the Document Approval Record located on the last page of this report.

GLP COMPLIANCE STATEMENT

This portion of the study was conducted in compliance with Good Laboratory Practice for Nonclinical Laboratory Studies regulations as set forth in the Code of Federal Regulations (21 CFR Part 58).

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OTHER STUDY PERSONNEL

The following study personnel were involved in the conduct of this study:

Peer Review Pathologist:	(b) (6)

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1. INTRODUCTION

BNT162b2 (Version 9 [V9]) and BNT162b3c are candidate COVID-19 vaccines, which consist of an LNP-encapsulated RNA encoding the SARS-CoV-2 spike protein or its derivatives, were administered intramuscularly (IM) once weekly for a total of 3 doses to male and female Wistar Han (Crl:WI[Han]) rats. Animals received the vehicle or test article at doses of 30 µg RNA/dosing day followed by a 3-week recovery phase. Group designations and doses are indicated in the table below.

Experimental Design				
Group Number	Test Article or Vehicle Dose (µg RNA/Dose Day)	Dose Volume (µL/injection site)[a]	Animal Numbers Males	Females
1	0[b]	60	1-15	46-60
2	30[c]	60	16-30	61-75
3	30[d]	60	31-45	76-90

a. Each animal received a single injection on each dose day.
b. Sterile saline.
c. BNT162b2(V9).
d. BNT162b3c.

Doses were administered by a single intramuscular injection (60 µL) on each dosing day (Days 1, 8, and 15) into the left hindlimb quadriceps muscle.

The first 10 animals/sex/group, by ascending animal order, were designated for necropsy at the end of the dosing phase (Day 17). The remaining animals were retained for the recovery phase.

2. MATERIALS AND METHODS

Necropsy, organ weights, macroscopic examination, tissue collection, tissue processing, and slide preparation were completed at the Testing Facility (Pfizer DSRD, Groton, CT) and microscopic examination and peer review were completed at the Test Site (Pfizer DSRD, Pearl River, NY).

2.1. Postmortem Observations

2.1.1. Euthanasia

Animals were euthanized by gas anesthesia (isoflurane) followed by exsanguination.

2.1.2. Necropsy

2.1.2.1. Scheduled Necropsy

Animals from the dosing phase were fasted overnight and euthanized on Dosing Phase Day 17, 2 days after the last dose (first 10 animals/sex/group). Animals from the recovery phase were fasted overnight and euthanized on Recovery Phase Day 22 (remaining 5 animals/sex/group). Complete necropsies, tissue collection, organ weights, and macroscopic tissue evaluation were performed on all animals. Necropsy included macroscopic examination of the external surface of the body, the thoracic and abdominal cavities and their contents, and the collection of all protocol-defined tissues.

2.1.2.2. Tissue Collection, Organ Weights and Tissues Processed for Slide Preparation – Dosing Phase

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Artery, Aorta		X	X	X
Bone Marrow, Sternum		X	X	X
Bone, Sternum		X	X	X
Brain	X	X	X	X
Cervix		X	X	X
Epididymis	X	X	X	X
Esophagus		X	X	X
Eye		X	X	X
Gland, Adrenal	X	X	X	X
Gland, Harderian		X	X	X
Gland, Lacrimal (Extraorbital)		X	X	X
Gland, Mammary		X	X	X
Gland, Parathyroid		X	X	X
Gland, Pituitary		X	X	X
Gland, Prostate	X	X	X	X
Gland, Salivary		X	X	X
Gland, Seminal Vesicle		X	X	X
Gland, Thyroid		X	X	X
Gut-Associated Lymphoid Tissue		X	X	X
Heart	X	X	X	X
Joint		X	X	X
Kidney	X	X	X	X
Large Intestine, Cecum		X	X	X
Large Intestine, Colon		X	X	X
Larynx				

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Liver	X	X	X	X
Lung		X	X	X
Lymph Node, Draining		X	X	X
Lymph Node, Inguinal		X	X	X
Lymph Node, Mesenteric		X	X	X
Macroscopic Findings		X	X	X
Muscle, Skeletal		X	X	X
Nerve, Optic		X	X	X
Nerve, Peripheral		X	X	X
Ovary	X	X	X	X
Oviduct		X	X	X
Pancreas		X	X	X
Site, Injection		X	X	X
Skin		X	X	X
Small Intestine, Duodenum		X	X	X
Small Intestine, Ileum		X	X	X
Small Intestine, Jejunum		X	X	X
Spinal Cord		X	X	X
Spleen	X	X	X	X
Stomach		X	X	X
Testis	X	X	X	X
Thymus	X	X	X	X
Tongue		X	X	X
Trachea		X	X	X
Ureter		X	X	X
Urinary Bladder		X	X	X
Uterus		X	X	X
Vagina		X	X	X

2.1.2.3. Tissue Collection, Organ Weights and Tissues Processed for Slide Preparation – Recovery Phase

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Artery, Aorta				
Bone Marrow, Sternum		X	X	X
Bone, Sternum				
Brain	X			

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Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Cervix				
Epididymis	X			
Esophagus				
Eye				
Gland, Adrenal	X			
Gland, Harderian				
Gland, Lacrimal (Extraorbital)				
Gland, Mammary				
Gland, Parathyroid				
Gland, Pituitary				
Gland, Prostate	X			
Gland, Salivary				
Gland, Seminal Vesicle				
Gland, Thyroid				
Gut-Associated Lymphoid Tissue				
Heart	X			
Joint		X	X	X
Kidney	X			
Large Intestine, Cecum				
Large Intestine, Colon				
Larynx				
Liver	X	X	X	X
Lung				
Lymph Node, Draining		X	X	X
Lymph Node, Inguinal		X	X	X
Lymph Node, Mesenteric				
Macroscopic Findings		X	X	X
Muscle, Skeletal		X	X	X
Nerve, Optic				
Nerve, Peripheral				
Ovary	X			
Oviduct				
Pancreas				
Site, Injection		X	X	X
Skin				
Small Intestine, Duodenum				
Small Intestine, Ileum				
Small Intestine, Jejunum				
Spinal Cord				
Spleen	X	X	X	X

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Stomach				
Testis	X			
Thymus	X			
Tongue				
Trachea				
Ureter				
Urinary Bladder				
Uterus				
Vagina				

2.1.3. Organ Weights

Designated organs from animals in Groups 1-3 were weighed at scheduled necropsy. Organ-to-body weight and organ-to-brain weight ratios were calculated.

2.1.4. Tissue Processing

Representative samples of collected tissues were fixed in 10% neutral buffered formalin except for eye with optic nerve attached (Davidson's), and testis and epididymis (modified Davidson's). All tissues processed for slide preparation were stained with hematoxylin and eosin.

2.1.5. Microscopic Examination

For the dosing phase, all tissues (excluding larynx) collected from all dosing phase animals were examined microscopically. For the recovery phase, tissues (bone marrow sternum, joint, liver, draining lymph node, inguinal lymph node, injection site, and spleen) from recovery animals in the control group and test article dose groups were examined microscopically. Microscopic findings were graded on a scale of 1 to 5 as minimal, mild, moderate, marked, or severe; findings not graded were listed as present.

2.1.6. Peer Review

Following completion of the tissue evaluation by the Anatomic Pathologists, a peer review evaluation was performed by another Pfizer Pathologist.

2.2. Statistical Analysis

Statistical analyses of organ weight data were conducted in Pristima with the methods outlined below. All analyses were performed separately for each sex.

Descriptive statistics were generated for each parameter and group at each scheduled sampling time or each time interval. Statistical tests were conducted at the 5% and 1% significance levels.

Analyses of organ weight parameters were done on measurements collected for each animal at the scheduled sampling times or time intervals. In addition, organ weight to body weight and organ weight to brain weight ratios were analyzed.

A nonparametric (rank-transform) one way analysis of variance (ANOVA) on all groups was conducted, with two-sided pairwise comparisons of Groups 2 and 3 to Group 1 using Dunnett's test. Average ranks were assigned to ties.

3. DATA ACQUISITION

Pristima Preclinical Data Management Suite (Version 7.4.3) was used to record pathology data.

4. DATA MANAGEMENT AND ARCHIVES

Raw data, documentation, protocol and amendments, final report, and any specimens generated at the Test Facility as the result of the study are retained at Pfizer, Groton, CT.

Materials including raw data and documents electronically archived are retained in the Pfizer OpenLab archive system or locked and retained in the source computerized system, as defined per SOP.

Materials are retained in accordance with the Enterprise Records Retention Schedule.

5. RESULTS

5.1. Unscheduled Euthanasia and Deaths

All animals survived until scheduled necropsy.

5.2. Postmortem Observations

5.2.1. Organ Weights

Group mean organ weight data are presented in Table 9. Individual animal organ weight data are included in Appendix 10. Results indicated below are test article group mean values compared with control group mean values.

Dosing Phase

Test article-related organ weight changes included higher absolute and relative (to body and brain weight) spleen weights in males and females administered BNT162b2 (V9) or BNT162b3c.

Higher group mean absolute and relative spleen weights were noted at 30 µg RNA/dosing day in BNT162b2 (V9) administered males (1.29x – 1.42x) and females (1.55x – 1.62x) and BNT162b3c administered males (1.34x – 1.52x) and females (1.41x – 1.47x) relative to control group means.

Text Table 1. Ratios of Test Article-Related Mean Absolute and Relative (To Body Weight and Brain Weight) Organ Weights Compared with Mean Controls

		Males			Females		
Dose (µg RNA/dosing day)		0	30[d]	30[e]	0	30[d]	30[e]
		Mean	Ratio		Mean	Ratio	
Spleen	Absolute (g)	0.5951	**1.29x**	**1.34x**	0.4382	**1.55x**	**1.41x**
	OW:BW[a]	0.2008	**1.42x**	**1.52x**	0.2202	**1.59x**	**1.47x**
	OW:BN[b]	0.3120	**1.29x**	**1.34x**	0.2353	**1.62x**	**1.43x**
Brain[c]	Absolute (g)	1.9061	1.01x	1.00x	1.8610	0.96x	0.99x
	OW:BW[a]	0.6449	1.10x	1.13x	0.9383	0.98x	1.02x
Terminal BW[c]	Absolute (g)	296.06	0.92x	0.89x	198.73	0.98x	0.97x

BN = Brain weight; BW = Body weight; g = grams OW = Organ weight.
a. Organ weight relative to terminal body weight.
b. Organ weight relative to brain weight.
c. Included for evaluating organ to brain weight and body weight ratios.
d. BNT162b2(V9)
e. BNT162b3c
Bold = test article related.

Recovery Phase

No test article-related organ weight changes were noted for either test article.

The spleen weights (absolute and relative to brain weight) were statistically increased in males administered BNT162b2 (V9). A similar change was not observed in females administered BNT162b2 (V9). A microscopic correlate was not identified in males (ie, no evidence of increased cellularity of hematopoietic cells), although one male had minimally

increased cellularity of germinal centers in the spleen. A similar increase in spleen weight was not identified in either males or females administered BNT162b3c. Therefore, the higher spleen weights in recovery males administered BNT162b2 (V9) was considered incidental and unrelated to vaccine administration, consistent with full recovery of the higher spleen weights observed at the end of dosing phase.

Other statistically significant or apparent alterations in mean absolute or relative organ weights were not test article-related because they did not occur in a dose-related pattern, were of low magnitude, lacked a macroscopic or microscopic correlate, occurred only in relative data, or occurred only in absolute data, but lost significance when evaluated relative to brain or body weight.

5.2.2. Macroscopic Findings

An incidence summary of macroscopic observations is presented in Table 10. Individual animal macroscopic observations are included in Appendix 11.

Dosing Phase

Test article-related macroscopic findings included large draining lymph nodes (abnormal size, enlarged) and dark/pale and/or firm injection sites (abnormal color, dark/pale and/or abnormal consistency, firm) in animals administered BNT162b2 (V9) or BNT162b3c, and large spleen and inguinal lymph nodes (abnormal size, enlarged) in animals administered BNT162b3c.

The macroscopic observation of large draining lymph nodes was present in BNT162b2 (V9) administered males and females and BNT162b3c administered females; large inguinal lymph nodes were observed in BNT162b3c administered females; pale/dark injection sites were observed in BNT162b2 (V9) administered males and females and BNT162b3c administered males; and firm injection sites were observed in BNT162b2 (V9) or BNT162b3c administered males and females. The macroscopic observation of enlarged spleen was limited to a single BNT162b3c administered female.

Text Table 2. Group Incidences of Test Article-Related Macroscopic Findings

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Number Examined	10	10	10	10	10	10
Lymph Node, Draining						
Abnormal size, enlarged	-	1	-	-	1	4
Lymph Node, Inguinal						

Text Table 2. Group Incidences of Test Article-Related Macroscopic Findings - Cont'd

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Number Examined	10	10	10	10	10	10
Abnormal size, enlarged	1[a]	-	-	-	-	2
Site, Injection						
Abnormal color, dark/pale	-	2	1	1	3	-
Abnormal consistency, firm	-	2	2	-	4	7
Spleen						
Abnormal size, enlarged	-	-	-	-	-	1

- = No finding present.
a. = No microscopic correlates.
b. BNT162b2(V9).
c. BNT162b3c.

Recovery Phase

Test article-related macroscopic findings observed at the end of recovery phase were limited to large draining lymph nodes (abnormal size, enlarged) in one male administered BNT162b2 (V9) and 1 female administered BNT162b3c and large inguinal lymph nodes (abnormal size, enlarged) in 1 female administered BNT162b3c, indicating a partial recovery of these findings. Pale/dark and/or firm injection sites and enlarged spleen were not observed at the end of recovery phase in BNT162b2 (V9) or BNT162b3c administered males and females, indicating a complete recovery of these findings.

Text Table 3. Group Incidences of Test Article-Related Macroscopic Findings

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[a]	30[b]	0	30[a]	30[b]
Number Examined	5	5	5	5	5	5
Lymph Node, Draining						
Abnormal size, enlarged	-	1	-	-	-	1
Lymph Node, Inguinal						
Abnormal size, enlarged	-	-	-	-	-	1

- = No finding present.
a. BNT162b2(V9).
b. BNT162b3c.

The remaining macroscopic findings were not test article-related effects because they were consistent with spontaneously occurring findings, the findings were distributed randomly

among groups, or their appearance was similar to findings in controls from this and/or previous studies.

5.2.3. Microscopic Findings

An expanded incidence summary of microscopic observations is presented in Table 11. Individual animal microscopic observations are included in Appendix 11.

Dosing Phase

Organs with test article-related microscopic findings included the injection site (mixed cell inflammation and edema), draining and inguinal lymph nodes (increased cellularity, plasma cells and germinal centers), liver (hepatocellular vacuolation), spleen (increased cellularity, hematopoietic cells and germinal centers), and bone marrow (increased cellularity, hematopoietic cells) in both males and females administered BNT162b2 (V9) or BNT162b3c. Inflammation at the injection site and increased cellularity of germinal centers in the lymph nodes were also observed in control animals but the incidence and/or severity was/were low and within the limits of the expected normal response to intramuscular injection of saline in these animals.

Text Table 4. Group Incidences (with Severities) of Test Article-Related Microscopic Findings

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Site, Injection[a]	10	10	10	10	10	10
Inflammation	4	10	10	5	10	10
Minimal (Grade 1)	4	-	-	5	-	-
Mild (Grade 2)	-	7	5	-	7	9
Moderate (Grade 3)	-	3	5	-	3	1
Edema	-	9	9	-	10	10
Mild (Grade 2)	-	8	8	-	9	9
Moderate (Grade 3)	-	1	1	-	1	1
Lymph Node, Draining[a]	10	9	10	10	10	10
Increased cellularity, Plasma cell	-	7	8	-	9	7
Minimal (Grade 1)	-	1	4	-	1	1
Mild (Grade 2)	-	4	3	-	1	5
Moderate (Grade 3)	-	2	1	-	7	1
Increased cellularity, Germinal center	2	6	8	2	5	6
Minimal (Grade 1)	1	2	2	1	3	4
Mild (Grade 2)	1	4	6	1	2	2
Lymph Node, Inguinal[a]	9	10	10	10	10	10
Increased cellularity, Plasma cell	-	1	1	-	2	4

Text Table 4. Group Incidences (with Severities) of Test Article-Related Microscopic Findings - Continued

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Minimal (Grade 1)	-	1	1	-	2	4
Increased cellularity, Germinal center	1	5	6	1	6	9
Minimal (Grade 1)	-	1	1	1	3	6
Mild (Grade 2)	1	4	5	-	3	3
Liver[a]	10	10	10	10	10	10
Vacuolation, Hepatocyte; Periportal	-	5	7	-	10	7
Minimal (Grade 1)	-	5	7	-	10	7
Spleen[a]	10	10	10	10	10	10
Increased cellularity, hematopoietic cell	-	10	10	-	9	10
Minimal (Grade 1)	-	10	10	-	9	10
Increased cellularity, Germinal center	-	5	5	-	6	5
Minimal (Grade 1)	-	5	5	-	6	5
Bone marrow, Sternum[a]	10	10	10	10	10	10
Increased cellularity, hematopoietic cell	-	10	10	-	10	10
Minimal (Grade 1)	-	10	10	-	10	10

- = No finding present.
a. Number examined.
b. BNT162b2(V9).
c. BNT162b3c.

Mixed cell inflammation at the injection site was characterized by large numbers of neutrophils with fewer plasma cells, macrophages, and lymphocytes admixed with abundant pale eosinophilic fluid (edema) and small amounts of cellular debris, fibrin, and hemorrhage. Inflammatory cells frequently infiltrated and expanded the epimysium, perimysium, and endomysium and separated and surrounded the myofibers and/or blood vessels in the skeletal muscle. Occasionally, inflammatory cells extended into the subcutaneous tissue/dermis of the overlying skin and into the extra-capsular tissue of the joint.

Increased cellularity in germinal center was observed in the lymph nodes and spleen compared to controls.

Increased cellularity of plasma cells in the lymph nodes was characterized by infiltration of variable numbers of plasma cells in the cortical, medullary, and subcapsular sinuses. Plasma cells were immature in appearance and were interpreted to likely represent plasmablasts.

Increased cellularity of hematopoietic cells in the bone marrow and spleen was characterized by increased hematopoietic precursor cells (primarily myeloid precursors) within the bone marrow or splenic red pulp compared to controls.

Vacuolation of the periportal hepatocytes in the liver was characterized by small clear round membrane bound structures within cytoplasm of these cells.

Recovery Phase

Test article-related microscopic findings noted at the end of the dosing phase including edema at the injection site, hepatocellular vacuolation in the liver, 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, indicating a complete recovery of these findings. Inflammation at the injection site was characterized by mostly lymphocytes and plasma cells with few neutrophils (indicating partial recovery) and no edema (full recovery). However, increased cellularity of plasma cells and germinal centers in the draining and inguinal lymph nodes, and increased cellularity of the germinal centers in the spleen partially recovered, as the incidence and/or severity of these findings were lower in recovery phase animals as compared to 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 plasmablasts 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 BNT162b3c. This finding was considered indicative of a reparative process (consequence of phagocytosis), which can be seen following inflammatory reactions at the injection sites.

Text Table 5. Group Incidences (with Severities) of Test Article-Related Microscopic Findings

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Site, Injection[a]	5	5	5	5	5	5
Inflammation	-	5	5	-	5	5
Minimal (Grade 1)	-	5	5	-	5	5
Lymph Node, Draining[a]	4	5	5	5	5	5
Increased cellularity, Plasma cell	-	4	5	-	4	3
Minimal (Grade 1)	-	4	5	-	4	3
Increased cellularity, Germinal center	-	4	4	1	3	5
Minimal (Grade 1)	-	3	2	1	2	4
Mild (Grade 2)	-	1	2	-	1	1
Infiltration, Macrophage	-	3	4	-	3	4
Minimal (Grade 1)	-	2	2	-	1	1
Mild (Grade 2)	-	1	2	-	2	3

Text Table 5. Group Incidences (with Severities) of Test Article-Related Microscopic Findings - Continued

Finding	Males Dose (µg RNA/dosing day)			Females Dose (µg RNA/dosing day)		
	0	30[b]	30[c]	0	30[b]	30[c]
Lymph Node, Inguinal[a]	5	5	5	5	5	5
Increased cellularity, Plasma cell	-	-	-	-	-	1
Minimal (Grade 1)	-	-	-	-	-	1
Increased cellularity, Germinal center	2	3	2	2	1	3
Minimal (Grade 1)	2	3	2	2	1	3
Infiltration, Macrophage	-	-	1	-	-	1
Minimal (Grade 1)	-	-	1	-	-	1
Spleen[a]	5	5	5	5	5	5
Increased cellularity, Germinal center	-	1	1	-	2	2
Minimal (Grade 1)	-	1	1	-	2	2

- = No finding present.
a. Number examined.
b. BNT162b2(V9).
c. BNT162b3c.

The remaining microscopic findings were not test article-related effects because they were consistent with spontaneously occurring findings, the findings were distributed randomly among groups, or their appearance was similar to findings in controls from this and/or previous studies.

6. INTEGRATED SUMMARY AND DISCUSSION OF RESULTS

BNT162b2 (Version 9 [V9]) and BNT162b3c, candidate COVID-19 vaccines, were administered by intramuscular (IM) doses once weekly for a total of 3 doses to male and female Wistar Han (Crl:WI[Han]) rats at 30 µg RNA/dosing day followed by a 3-week recovery phase.

All test article-related pathology findings with both vaccine candidates were interpreted as nonadverse, as there was no evidence of systemic toxicity or clinical signs of illness or lameness.

At the end of the dosing phase, test article-related mixed cell inflammation (mild to moderate) and edema (mild to moderate) at the injection site were consistent with those typically associated with the IM administration of lipid nanoparticle (LNP)-encapsulated mRNA vaccines (Hassett et al, 2019). These findings correlated with macroscopic observations of abnormal color (dark/pale) and consistency (firm). At the end of the 3-week recovery phase, full recovery occurred for macroscopic findings of pale/dark and firm

injection sites and the microscopic finding of edema, whereas partial recovery occurred for inflammation at the injection sites.

At the end of the dosing phase, test article-related findings in the lymph nodes (increased cellularity of plasma cells [minimal to moderate] and germinal centers [minimal to mild]), spleen (increased cellularity of hematopoietic cells [minimal] and germinal centers [minimal]), and the bone marrow (minimal increased cellularity of hematopoietic cells) were secondary to immune activation and/or inflammation at the injection site. The presence of plasma cells (interpreted as plasmablasts) in the draining and inguinal lymph nodes was interpreted to reflect a robust immunological response to the vaccines. These observations correlated with macroscopic observations of abnormal size (enlarged) in the lymph nodes and spleen and increased splenic weights. At the end of the 3-week recovery phase, full recovery occurred for higher spleen weights, macroscopic finding of enlarged spleen, and microscopic findings of increased cellularity of hematopoietic cells in the spleen and bone marrow, whereas partial recovery occurred for macroscopic findings of enlarged draining and inguinal lymph nodes, microscopic findings of increased cellularity of plasma cells and germinal centers in the draining and inguinal lymph nodes, and increased cellularity of the germinal centers in the spleen.

At the end of the dosing phase, test article-related microscopic finding of minimal portal hepatocyte vacuolation was not associated with hepatic tissue damage or liver enzyme alterations. This change might be related to hepatic clearance of the pegylated lipid in the LNP (Ivens et al, 2015). At the end of 3-week recovery phase, this finding was completely recovered.

In conclusion, administration of BNT162b2 (Version 9 [V9]) or BNT162b3c, at 30 µg RNA/dosing day by intramuscular (IM) administration once weekly to Wistar Han rats for 3 weeks did not result in any adverse findings. All test article-related effects were nonadverse, and except for hepatocyte vacuolation, effects were consistent with expected immune responses to vaccines and/or secondary to inflammation. Full or partial recovery occurred in both males and females administered BNT162b2 (V9) or BNT162b3c for all findings by the end of the recovery phase.

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Medical Quality Assurance

Quality Assurance Statement

Title: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study: 20GR142

In accordance with Pfizer policies and Medical Quality Assurance procedures for Good Laboratory Practice (GLP), the conduct of this portion of this study has been inspected and/or audited as follows.

Phase Inspected	Audit/Inspection Date GMT	Reporting Date GMT
Test Site Protocol Review	24-Jun-2020 to 25-Jun-2020	25-Jun-2020
Protocol Amendment #1	02-Jul-2020 to 02-Jul-2020	02-Jul-2020
Protocol Amendment #4	04-Sep-2020 to 04-Sep-2020	04-Sep-2020
Report: Clinical Pathology	20-Oct-2020 to 30-Oct-2020	03-Nov-2020
Report: Pathology	20-Oct-2020 to 30-Oct-2020	03-Nov-2020

In addition Routine Facility and Process audits are conducted in accordance with MQA SOPs and Site Monitoring Plans.

(b) (6)

Document Approval Record

Document Name:	Anatomic Pathology
Document Title:	20GR142 Anatomic Pathology

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	09-Nov-2020 17:49:26	Quality Assurance Approval
	09-Nov-2020 17:55:46	Scientific Review
	09-Nov-2020 19:54:09	Author Approval

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Non-GMP CoA

Material not for human use
Version 3

Product: **CoVVAC**
Batch: RBP020.2LNP
Lot: CoVVAC/270320

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/016)	
RNA integrity	CE (223/SOP/016)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 content	HPLC-CAD (222/SOP/044)	
ALC-0159 content	HPLC-CAD (222/SOP/044)	
DSPC content	HPLC-CAD (222/SOP/044)	
Cholesterol content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 09.04.20 (b) (6)

Date: 09.04.20

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Non-GMP CoA

Material not for human use
Version 2

Product: **CorVac BNT162b3c**
Batch: RBP020.8 LNP
Lot: BCV/040620

Test	Method	Result
Appearance	Visual Inspection (224/SOP/011)	(b) (4)
RNA identity	CE (223/SOP/015)	
RNA integrity	CE (223/SOP/015)	
RNA content	Ribogreen Assay (221/SOP/018)	
RNA encapsulation	Ribogreen assay +/- LNP disruption (221/SOP/018)	
ALC-0315 identification and content	HPLC-CAD (222/SOP/044)	
ALC-0159 identification and content	HPLC-CAD (222/SOP/044)	
DSPC identification and content	HPLC-CAD (222/SOP/044)	
Cholesterol identification and content	HPLC-CAD (222/SOP/044)	
Particle size (Z_{avg})	Dynamic light scattering (224/SOP/002)	
Polydispersity index (PDI)	Dynamic light scattering (224/SOP/002)	
pH	pH (224/SOP/016)	
Osmolality	Freezing point depression (224/SOP/009)	
Endotoxins/Pyrogens	Turbidimetric, kinetic LAL assay (Ph.Eur. 2.6.14/ USP<85>)	
Bioburden	Membrane filtration method 225/SOP/001	

Store at: -70°C

Date: 03.07.2020

Date: 03.07.2020

(b) (6)

Medical Quality Assurance

Quality Assurance Statement

Title: 17-DAY INTRAMUSCULAR TOXICITY STUDY OF BNT162B2 (V9) AND BNT162B3C IN WISTAR HAN RATS WITH A 3-WEEK RECOVERY

Study: 20GR142

In accordance with Pfizer policies and Medical Research Quality Assurance procedures for Good Laboratory Practice (GLP), the conduct of this study has been inspected and/or audited as follows. The Individual Quality Assurance Statement for study phase(s) conducted at other site(s) are contained within this report.

Phase Inspected	Audit/Inspection Date GMT	Reporting Date GMT
Protocol Review	29-Jun-2020 to 29-Jun-2020	30-Jun-2020
Protocol Amendment #1	07-Jul-2020 to 07-Jul-2020	07-Jul-2020
In-life: Dosing	13-Jul-2020 to 14-Jul-2020	16-Jul-2020
In-life: Dosing	13-Jul-2020 to 14-Jul-2020	16-Jul-2020
Protocol Amendment #2	16-Jul-2020 to 16-Jul-2020	16-Jul-2020
In-life: Clin Path Blood Collection	22-Jul-2020 to 23-Jul-2020	24-Jul-2020
In-life: Necropsy	22-Jul-2020 to 22-Jul-2020	24-Jul-2020
Protocol Amendment #3	28-Jul-2020 to 28-Jul-2020	03-Aug-2020
Protocol Amendment #4	08-Sep-2020 to 08-Sep-2020	08-Sep-2020
Report: Nonclinical Study	14-Oct-2020 to 03-Nov-2020	03-Nov-2020

In addition Routine Facility and Process audits are conducted in accordance with MQA SOPs and Site Monitoring Plans.

(b) (6)

Pfizer Confidential

Document Approval Record

Document Name:	Study 20GR142 - 17-Day Intramuscular Toxicity Study of BNT162B2 (V9) and BNT162B3C in Wistar Han Rats with a 3-Week Recovery
Document Title:	Final Report - 17-Day Intramuscular Toxicity Study of BNT162B2 (V9) and BNT162B3C in Wistar Han Rats with a 3-Week Recovery

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Article

Respiratory disease in rhesus macaques inoculated with SARS-CoV-2

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An outbreak of a novel coronavirus, named SARS-CoV-2, causing respiratory disease and a ~2% case fatality rate started in Wuhan, China in December 2019[1,2]. Following unprecedented global spread[3], the World Health Organization declared COVID-19 a pandemic on March 11, 2020. Although data on disease in humans are emerging at a steady pace, certain aspects of the pathogenesis of SARS-CoV-2 can only be studied in detail in animal models, where repeated sampling and tissue collection is possible. Here, we show that SARS-CoV-2 causes respiratory disease in infected rhesus macaques, with disease lasting 8-16 days. Pulmonary infiltrates, a hallmark of human disease, were visible in lung radiographs. High viral loads were detected in swabs from the nose and throat of all animals as well as in bronchoalveolar lavages; in one animal we observed prolonged rectal shedding. Taken together, the rhesus macaque recapitulates moderate disease observed in the majority of human cases. The establishment of the rhesus macaque as a model of COVID-19 will increase our understanding of the pathogenesis of this disease and will aid development and testing of medical countermeasures.

SARS-CoV-2 infection in humans can be asymptomatic or result in mild to fatal Coronavirus Disease 2019 (COVID-19)[4–6]. Patients with COVID-19 pneumonia presented mainly with fever, fatigue, dyspnea and cough[7–9]. Rapidly progressing pneumonia, with bilateral opacities on x-ray or patchy shadows and ground glass opacities by CT scan were observed in COVID-19 patients[2,6,10]. Older patients with comorbidities are at highest risk for adverse outcome of COVID-19[5,7]. SARS-CoV-2 has been detected in upper and lower respiratory tract samples from patients, as well as feces and blood, but not in urine[5,11–13].

Non-human primate models that recapitulate aspects of human disease are essential for our understanding of the pathogenic processes involved in severe respiratory disease and the development of medical countermeasures such as vaccines and antivirals.

Clinical, respiratory disease

Eight adult rhesus macaques were inoculated with SARS-CoV-2 isolate nCoV-WA1-2020[14]. On day 1 post inoculation (dpi), all animals showed changes in respiratory pattern and piloerection, as reflected in their clinical scores (Fig. 1a). Other observed signs of disease included reduced appetite, hunched posture, pale appearance and dehydration (Extended Data Table 1). Coughing was occasionally heard in the room where animals were housed but could not be pinpointed to individual animals. Disease signs persisted for more than a week, with all animals completely recovered between 9 and 17 dpi (Fig. 1a and Table S1). Weight loss was observed in all animals (Fig. 1b); body temperatures spiked on 1 dpi but returned to normal levels thereafter (Fig. 1c). Under anesthesia, the animals did not show increased respiration; however, all animals showed irregular respiration patterns (Fig. 1d). Radiographs showed pulmonary infiltrates in all animals starting on 1 dpi with mild pulmonary infiltration primarily in the lower lung lobes. By 3 dpi, progression of mild pulmonary infiltration was noted into other lung lobes although still primarily in the caudal lung lobes (Fig. 1e). In one animal, pulmonary infiltrates were observed from 1-12 dpi (Extended Data Fig. 1).

Hematologic analysis of blood collected during clinical exams showed evidence of a stress leukogram[15] by 1 dpi in the majority of animals (Extended Data Fig. 2). Lymphocytes and monocytes returned to baseline after 1 dpi. Neutrophils decreased in all animals by 3 dpi and continued to decline through 5 dpi; neutropenia was observed in 2 of 4 animals. On 1 dpi, decreased hematocrit, red blood cell counts and hemoglobin were observed in all animals (Extended Data Fig. 2). In addition, reticulocyte percentages and counts decreased. At 5 dpi, two of four animals had a normocytic, normochromic non-regenerative anemia consistent with anemia of critical illness; animals did not return to their original baselines by 21 dpi. Blood chemistry analysis revealed no values outside normal range (Supplementary Information Table S2).

Serum was analyzed for changes in cytokine and chemokine levels at different time points after inoculation. Statistically significant changes were only observed on 1 dpi, with increases in IL1ra, IL6, IL10, IL15, MCP-1, MIP-1b, and on 3 dpi a small but statistically significant decrease in TGFα was observed (Extended Data Fig. 3). Although changes occurred

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in the levels of some of these cytokines later after inoculation, these were not statistically significant (Extended Data Fig. 3).

High viral loads in respiratory samples

Virus shedding was highest from the nose (Fig. 2a); virus could be isolated from swabs collected on 1 and 3 dpi, but not thereafter. Viral loads were high in throat swabs immediately after inoculation but were less consistent than nose swabs thereafter; in one animal throat swabs were positive on 1 and 10 dpi but not in between (Fig. 2a). One animal showed prolonged shedding of viral RNA in rectal swabs; infectious virus could not be isolated from these swabs (Fig. 2a) and intestinal tract disease (e.g. diarrhea) was not observed. Urogenital swabs remained negative in all animals throughout the study. On 1, 3 and 5 dpi bronchoalveolar lavages (BAL) were performed on the 4 animals in the group euthanized on 21 dpi. High viral loads were detected in BAL fluid in all animals on all three time points; infectious virus could only be isolated in BAL fluid collected on 1 and 3 dpi (Fig. 2b). No viral RNA could be detected in blood (Fig. 2c) or urine (Fig. 2d).

Interstitial pneumonia

On 3 and 21 dpi, one group of 4 animals was euthanized and necropsies were performed.

On 3 dpi, varying degrees of gross lung lesions were observed in all animals (Fig. 3a and c). By 21 dpi, gross lesions were still visible in the lungs of 2 of 4 animals (Fig. 3b and c). Additionally, all animals had an increased lung weight:body weight ratio (Fig. 3d) as compared to healthy rhesus macaques, indicative of pulmonary edema. Histologically, 3 of the 4 animals euthanized on 3 dpi developed some degree of pulmonary pathology. Lesions were multifocal (Extended Data Fig. 4a), mild to moderate, interstitial pneumonia that frequently centered on terminal bronchioles. The pneumonia was characterized by thickening of alveolar septae by edema fluid and fibrin and small to moderate numbers of macrophages and fewer neutrophils. Lungs with moderate changes also had alveolar edema and fibrin with formation of hyaline membranes. There was minimal type II pneumocyte hyperplasia. Occasionally, bronchioles showed necrosis, loss and attenuation of the epithelium with infiltrates of neutrophils, macrophages and eosinophils. Multifocally, there were perivascular infiltrates of small numbers of lymphocytes forming perivascular cuffs (Extended Data Figure 4b) and minimal to mild, multifocal hyperplasia of bronchiolar associated lymphoid tissue. Three of 4 animals on 3 dpi had fibrous adhesions of the lung to the pleura. Histologic evaluation showed these to be composed of mature collagen interspersed with small blood vessels; therefore, this is most likely a chronic change rather than related to SARS-CoV-2 infection. Minimal to mild inflammation was observed in the upper airways with multifocal squamous metaplasia of the respiratory epithelium with infiltration of small numbers of neutrophils (Extended Data Figure 5).

Immunohistochemistry using a mAb against SARS-CoV demonstrated viral antigen in small numbers of type I and II pneumocytes, as well as alveolar macrophages. Antigen-positive macrophages were detected in mediastinal lymph nodes of 3 of 4 animals (Fig. 3k). Interestingly, small numbers of antigen-positive lymphocytes and macrophages were also detected in the lamina propria of the intestinal tract of all 4 animals. In one animal, all collected tissues of the gastrointestinal tract showed these antigen-positive mononuclear cells (Extended Data Figure 6).

Ultrastructural analysis of lung tissue by transmission electron microscopy confirmed the histologic diagnosis of interstitial pneumonia. The alveolar interstitial space was greatly expanded by edema, fibrin, macrophages and neutrophils (Extended Data Figure 7a). The subepithelial basement membrane was unaffected and maintained a consistent thickness and electron density. Occasionally, type I pneumocytes are separated from the basement membrane by edema; the resulting space may contain virions. Affected type I pneumocytes are lined by small to moderate numbers of virions 90-160 nm in diameter with an electron dense core bound by a less dense capsid (Extended Data Figure 7b-e). Alveolar spaces adjacent to affected pneumocytes are filled with a granular, moderately electron dense material that is consistent with edema fluid.

Replication in the respiratory tract

All tissues (n=37) collected at necropsy were analyzed for the presence of viral RNA. On 3 dpi, high viral loads were detected in the lungs of all animals (Extended Data Fig. 8a); virus could be isolated from the lungs of all 4 animals at this time. Additionally, viral RNA could be detected in other samples throughout the respiratory tract (Extended Data Fig. 8), as well as in lymphoid and gastrointestinal tissues. Viral RNA could not be detected in major organs including the central nervous system. To distinguish viral RNA derived from respiratory secretions from active virus replication, all samples with presence of viral RNA were also tested for the presence of viral mRNA (Extended Data Fig. 8). Viral mRNA was detected in all respiratory tissues but could not in any but one of the gastrointestinal tissues, indicating that virus replication in these tissues seems unlikely, although we can't exclude it due to limited sample size. By 21 dpi, viral RNA, but not mRNA, could still be detected in tissues from all 4 animals (Extended Data Fig. 8g).

Serology

Serum was analyzed for the development of IgG against SARS-CoV spike in ELISA. By 10 dpi, all four animals had seroconverted to SARS-CoV-2 spike; neutralizing responses also started to appear at 10 dpi (Extended Data Figure 9). Interestingly, the animal with the lowest and latest neutralizing antibody response was the animal with prolonged viral shedding from the intestinal tract.

Discussion

COVID-19 clinical manifestations range from asymptomatic to mild to severe[5,6,8,9,13,16]. Patients present with influenza-like symptoms such as fever and shortness of breath and may develop pneumonia requiring mechanical ventilation and support in an intensive care unit[9]. Similar to SARS-CoV and MERS-CoV, comorbidities such as hypertension and diabetes play an important role in adverse outcome of COVID-19[8,17,18]. Advanced age and chronic conditions in particular are indicators of a negative outcome[5,7–9,16], conditions that were absent in our healthy rhesus macaques. An analysis of 1099 COVID-19 cases from China showed that approximately 5% of diagnosed patients developed severe pneumonia requiring ICU attendance, 2.3% required mechanical ventilation and 1.4% died[9]. The transient, moderate disease observed here in rhesus macaques is thus in line with the majority of human COVID-19 cases. Pulmonary infiltrates on radiographs, a hallmark of human infection[2,4,6,7,9,10,16], were observed in all macaques. The shedding pattern observed in rhesus macaques is strikingly similar to that observed in humans[11,12]. In humans, consistent high SARS-CoV-2 shedding was observed from the upper and lower respiratory tract, frequent intermediate shedding from the intestinal tract and sporadic detection in blood[5]. Similar to humans, shedding of SARS-CoV-2 continued after resolution of clinical symptoms and radiologic abnormalities[19]. Limited histopathology is available from COVID-19 patients[20,21]. Our analysis of the histopathological changes observed in the lungs of rhesus macaques, suggests that they resemble those observed with SARS-CoV and MERS-CoV[21–24], with regard to lesion type and cell tropism.

Serological responses in humans are not typically detectable before 6 days after symptom onset, with IgG titers between 100 and 10,000 observed after 12 to 21 days[25,26]. Neutralizing titers were generally between 20 – 160. This corresponds to the results in our rhesus

macaque model, where IgG responses were detected around 7-10 dpi. Seroconversion was not directly followed by a decline in viral loads, as observed in COVID-19 patients[25,26].

Taken together, the rhesus macaque model recapitulates COVID-19, with regard to virus replication and shedding, the presence of pulmonary infiltrates, histological lesions and seroconversion. This extensive dataset allows us to bridge between the rhesus macaques model and the disease observed in humans and to utilize this animal model to assess the efficacy of medical countermeasures.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2324-7.

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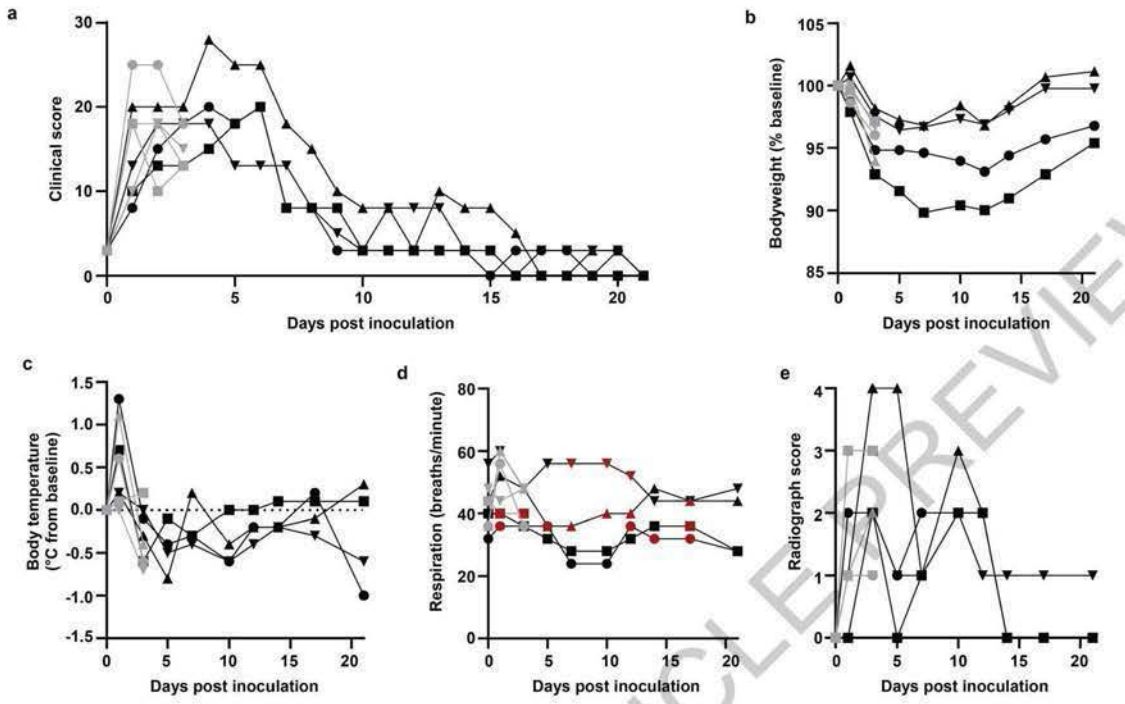

Fig. 1 | Rhesus macaques infected with SARS-CoV-2 develop respiratory disease. After inoculation with SARS-CoV-2, animals were observed for disease signs and scored according to a pre-established clinical scoring sheet (a). On clinical exams, body weight (b), and body temperature (c) were measured. Respiration rate was measured, and breathing pattern was recorded, with irregular respiration patterns indicated in red (d). Ventro-dorsal and lateral radiographs were taken on clinical exam days and scored for the presence of pulmonary infiltrates (0: normal; 1: mild interstitial pulmonary infiltrates; 2: moderate pulmonary infiltrates perhaps with partial cardiac border effacement and small areas of pulmonary consolidation; 3: severe interstitial infiltrates, large areas of pulmonary consolidation, alveolar patterns and air bronchograms). Individual lobes were scored and scores per animal per day totaled (e). Grey: animals euthanized 3 dpi; black: animals euthanized 21 dpi. Identical symbols have been used to denote identical animals throughout this manuscript.

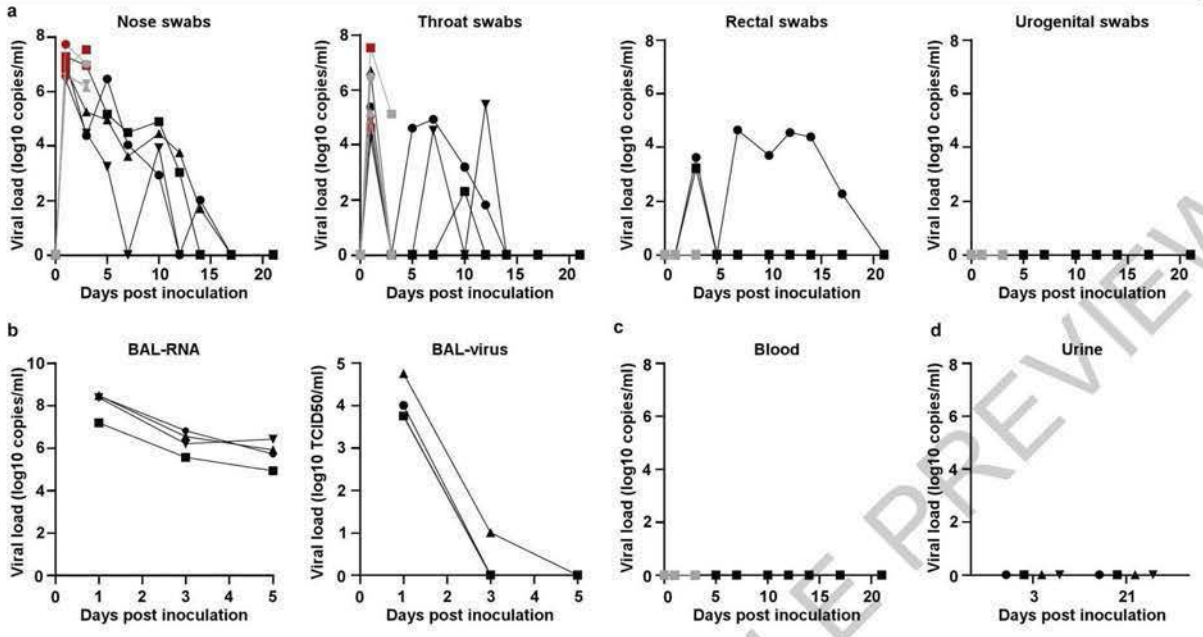

Fig. 2 | Viral loads in respiratory samples and bodily fluids. After inoculation, nose, throat, rectal and urogenital swabs were collected; viral loads in these samples were determined by qRT-PCR (a). On 1, 3, and 5 dpi, bronchoalveolar lavages were performed on the 4 animals remaining in the study through 21 dpi; viral loads and virus titers were determined in these samples. Viral loads were determined in blood collected during clinical exams (c) and urine collected at necropsy on 3 and 21 (d). Grey: animals euthanized 3 dpi; black: animals euthanized 21 dpi; red: virus was isolated from these samples. Identical symbols have been used to denote identical animals this manuscript.

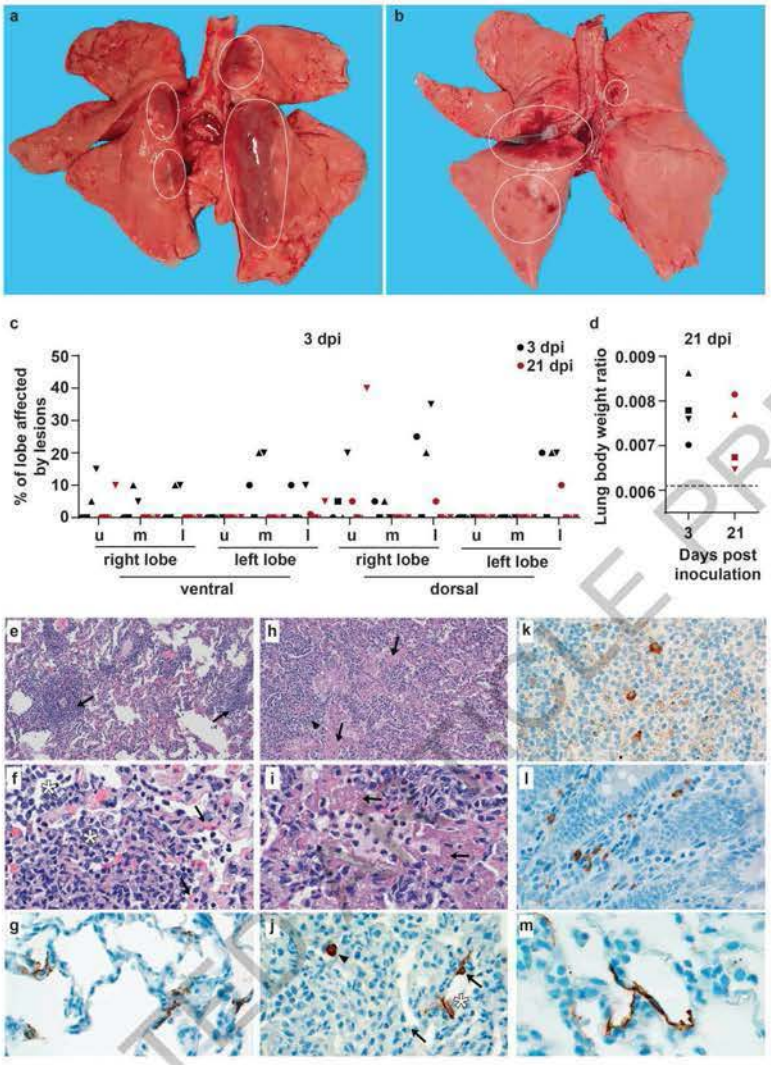

Fig. 3 | Pathological changes in rhesus macaques infected with SARS-CoV-2. Four rhesus macaques were euthanized on 3 and 21 dpi. Grossly, lungs showed focal areas of hilar consolidation and hyperemia (circles) on 3 dpi (a) and multifocal, random consolidation and hyperemia (circles) on 21 dpi (b). The percentage of the area of the lungs affected by gross lesions was estimated (c), and lung weight to bodyweight ratio was calculated. (d). The dotted line represents baseline ratio calculated from an in-house collection of rhesus macaque lung and bodyweights from animals with grossly normal lungs. Histological analysis was performed on tissues collected at 3 dpi (e-i). Tissue sections were collected from the same anatomical location for each animal; three tissue sections were prepared from each of the 6 lung lobes. In total, 18 lung sections were evaluated for each animal; representative images are displayed. (e) Pulmonary vessels surrounded by moderate numbers of lymphocytes and fewer macrophages (arrows). (f) Alveoli filled with small to moderate numbers of macrophages and neutrophils (asterisks). Adjacent alveolar interstitium (arrows) is thickened by edema, fibrin, neutrophils, lymphocytes and macrophages. (g) SARS-CoV-2 antigen detected by immunohistochemistry in type I pneumocytes. (h) Pulmonary vessels bounded by lymphocytes (arrowhead) and hyaline membranes (arrows) line alveolar spaces. (i) Hyaline membranes line alveoli (arrows). (j) SARS-CoV-2 antigen detected by immunohistochemistry in type I pneumocytes (asterisk) and type II pneumocytes (arrow) as well as alveolar macrophages (arrowheads). (k) SARS-CoV-2 antigen detected by immunohistochemistry in macrophages in a mediastinal lymph node. (l) SARS-CoV-2 antigen detected by immunohistochemistry in macrophages and lymphocytes in the lamina propria of the cecum. (m) SARS-CoV-2 detected by immunohistochemistry in type I pneumocytes. Magnification: e, h 100x; f, g, I, j, k, l 400x; m: 1000x. u: upper; m: middle; l: lower.

Methods

Ethics and biosafety statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIH and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, following the guidelines and basic principles in the NIH Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, United States Department of Agriculture and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. Rhesus macaques were housed in adjacent individual primate cages allowing social interactions, in a climate-controlled room with a fixed light-dark cycle (12-hr light/12-hr dark). Animals were monitored at least twice daily throughout the experiment. Commercial monkey chow, treats, and fruit were provided twice daily by trained personnel. Water was available ad libitum. Environmental enrichment consisted of a variety of human interaction, manipulanda, commercial toys, videos, and music. The Institutional Biosafety Committee (IBC) approved work with infectious SARS-CoV-2 strains under BSL3 conditions. Sample inactivation was performed according to IBC-approved standard operating procedures for removal of specimens from high containment.

Study design

To evaluate the use of rhesus macaques as a model for SARS-CoV-2, eight adult rhesus macaques (4 males, and4 females, age 4-6 years) were inoculated via a combination of intranasal (0.5ml per nostril), intratracheal (4ml), oral (1ml) and ocular (0.25ml per eye) of a $4x10^5$ TCID50/ml ($3x10^8$ genome copies/ml) virus dilution in sterile DMEM. The animals were observed twice daily for clinical signs of disease using a standardized scoring sheet (Supplementary Information Table S1); the same person assessed the animals throughout the study. The predetermined endpoint for this experiment was 3 days post inoculation (dpi) for one group of 4 animals, and 21 dpi for the remaining 4 animals. Animals were randomly assigned to a group for necropsy prior to the start of the experiment. Blinding was not used in this study since all animals were subjected to the same treatment. Clinical exams were performed on 0, 1, 3, 5, 7, 10, 12, 14, 17 and 21 dpi on anaesthetized animals. On exam days, clinical parameters such as bodyweight, body temperature and respiration rate were collected, as well as ventro-dorsal and lateral chest radiographs. Chest radiographs were interpreted by a board-certified clinical veterinarian. The following samples were collected at all clinical exams: nasal, throat, urogenital and rectal swabs, blood. The total white blood cell count, lymphocyte, neutrophil, platelet, reticulocyte and red blood cell counts, hemoglobin, and hematocrit values were determined from EDTA blood with the IDEXX ProCyte DX analyzer (IDEXX Laboratories). Serum biochemistry (albumin, AST, ALT, GGT, BUN, creatinine) was analyzed using the Piccolo Xpress Chemistry Analyzer and Piccolo General Chemistry 13 Panel discs (Abaxis). During clinical exams on 1, 3, and 5 dpi bronchoalveolar lavages were performed using 10ml sterile saline. Of note, repeated bronchoalveolar lavages do not induce lung damage when space 48 hrs apart[27,28]. After euthanasia, necropsies were performed. The percentage of gross lung lesions was scored by a board-certified veterinary pathologist and samples of the following tissues were collected: inguinal lymph node, axillary lymph node, cervical lymph node, salivary gland, conjunctiva, nasal mucosa, oropharynx, tonsil, trachea, all six lung lobes, mediastinal lymph node, right and left bronchus, heart, liver, spleen, pancreas, adrenal gland, kidney, mesenteric lymph node, stomach, duodenum, jejunum, ileum, cecum, colon, urinary bladder, reproductive tract (testes or ovaries depending on sex of the animal), bone marrow, frontal brain, cerebellum and brainstem. Histopathological analysis of tissue slides was performed by a board-certified veterinary pathologist blinded to the group assignment of the animals.

Virus and cells

SARS-CoV-2 isolate nCoV-WA1-2020 (MN985325.1)[14] (Vero passage 3) was kindly provided by CDC and propagated once in VeroE6 cells in DMEM (Sigma) supplemented with 2% fetal bovine serum (Gibco), 1 mM L-glutamine (Gibco), 50 U/ml penicillin and 50 µg/ml streptomycin (Gibco) (virus isolation medium). The used virus stock was 100% identical to the initial deposited genbank sequence (MN985325.1) and no contaminants were detected. VeroE6 cells were maintained in DMEM supplemented with 10% fetal calf serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin. VeroE6 cells were provided by Dr. Ralph Baric and were not authenticated in-house; mycoplasma testing is performed at regular intervals and no mycoplasma has been detected.

Quantitative PCR

RNA was extracted from swabs and BAL using the QiaAmp Viral RNA kit (Qiagen) according to the manufacturer's instructions. Tissues (30 mg) were homogenized in RLT buffer and RNA was extracted using the RNeasy kit (Qiagen) according to the manufacturer's instructions. For detection of viral RNA, 5 µl RNA was used in a one-step real-time RT-PCR E assay[29] using the Rotor-Gene probe kit (Qiagen) according to instructions of the manufacturer. In each run, standard dilutions of counted RNA standards were run in parallel, to calculate copy numbers in the samples. For detection of SARS-CoV-2 mRNA, primers targeting open reading frame 7 (ORF7) were designed as follows: forward primer 5'-TCCCAGGTAACAAACCAACC-3', reverse primer 5'-GCTCACAAGTAGCGAGTGTTAT-3', and probe FAM-ZEN-CTTGTAGATCTGTTCTCTAAACGAAC-IBFQ. 5 µl RNA was used in a one-step real-time RT-PCR using the Rotor-Gene probe kit (Qiagen) according to instructions of the manufacturer. In each run, standard dilutions of counted RNA standards were run in parallel, to calculate copy numbers in the samples.

Histopathology and immunohistochemistry

Histopathology and immunohistochemistry were performed on rhesus macaque tissues. After fixation for a minimum of 7 days in 10% neutral-buffered formalin and embedding in paraffin, tissue sections were stained with hematoxylin and eosin (HE). To detect SARS-CoV-2 antigen, immunohistochemistry was performed using an anti-SARS nucleocapsid protein antibody (Novus Biologicals) at a 1:250 dilution. This antibody was first tested on SARS-CoV-2 infected and uninfected Vero E6 cell pellets, showing specific staining with infected cells and no staining with uninfected cells. The antibody showed specific staining with infected experimental tissue and no staining with uninfected tissue from rhesus macaques. Infected tissue and cell pellet specimens showed no staining when run with Rabbit IgG controls (non-specific rabbit IgG substituted for primary antibody). Stained slides were analyzed by a board-certified veterinary pathologist.

Transmission electron microscopy. After fixation for 7 days with Karnovsky's fixative at 4 °C, excised tissues were post-fixed for 1 hour with 0.5% osmium tetroxide/0.8% potassium ferricyanide in 0.1 M sodium cacodylate, washed 3 x 5 minutes with 0.1M sodium cacodylate buffer, stained 1 hour with 1% tannic acid, washed with buffer and then further stained with2% osmium tetroxide in 0.1M sodium cacodylate and overnight with 1% uranyl acetate at 4 °C. Specimens were dehydrated with a graded ethanol series with two final exchanges in 100% propylene oxide before infiltration and final embedding in Embed-812/Araldite resin. Thin sections were cut with a Leica EM UC6 ultramicrotome (Leica, Vienna, Austria), prior to viewing at 120 kV on a Tecnai BT Spirit transmission electron microscope (Thermo fisher/FEI, Hillsboro, OR). Digital images were acquired with a Gatan Rio bottom mount digital camera system (Gatan Inc., Pleasanton, CA

and processed using Adobe Photoshop v. CC 2019 (Adobe Systems Inc, San Jose, CA).

Serum cytokine and chemokine analysis. Serum samples for analysis of cytokine/chemokine levels were inactivated with γ-radiation (2 MRad) according to standard operating procedures. Concentrations of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon (IFN)−γ, interleukin (IL)−1β, IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12/23 (p40), IL-13, IL-15, IL-17, MCP-1 and macrophage inflammatory protein (MIP)−1α, MIP-1β, soluble CD40-ligand (sCD40L), transforming growth factor-α, tumor necrosis factor (TNF)−α, vascular endothelial growth factor (VEGF) and IL-18 were measured on a Bio-Plex 200 instrument (Bio-Rad) using the Non-Human Primate Cytokine MILLIPLEX map 23-plex kit (Millipore) according to the manufacturer's instructions.

Serology

Sera were analyzed by SARS-CoV-2 spike protein (S) enzyme-linked immunosorbent assay (ELISA) as done previously for MERS-CoV[30]. Briefly, maxisorp (Nunc) plates were coated overnight with 100 ng/well S protein diluted in PBS[31] (a kind gift of Barney Graham, Vaccine Research Center, NIH) and blocked with blocker casein in PBS (Life Technologies). Sera were serially diluted in duplicate. SARS-CoV-2-specific antibodies were detected using anti-monkey IgG polyclonal antibody HRP-conjugated antibody (KPL), peroxidase-substrate reagent (KPL) and stop reagent (KPL). Optical density (OD) was measured at 405 nm. The threshold of positivity was calculated by taking the average of the day 0 values multiplied by 3.

For neutralization, sera were heat-inactivated (30 min, 56 °C) and two-fold serial dilutions were prepared in 2% DMEM. Hereafter, 100 $TCID_{50}$ of SARS-CoV-2 was added. After 60 min incubation at 37 °C, virus:serum mixture was added to VeroE6 cells and incubated at 37 °C and 5% CO2. At 5 dpi, cytopathic effect was scored. The virus neutralization titer is expressed as the reciprocal value of the highest dilution of the serum which still inhibited virus replication. All sera were analyzed in duplicate.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Data have been deposited in Figshare: https://doi.org/10.35092/yhjc.12026910.

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Author contributions VJM and EdW designed the study; VJM, FF, BW, NvD, LPP, JS, KMW, AO, JC, BB, VAA, RR, PH, GS, EF, DS and EdW acquired, analyzed and interpreted the data; VJM, PH, EF, DS and EdW wrote the manuscript. All authors have approved the submitted version.

Competing interests The authors declare no competing interests.

Additional information

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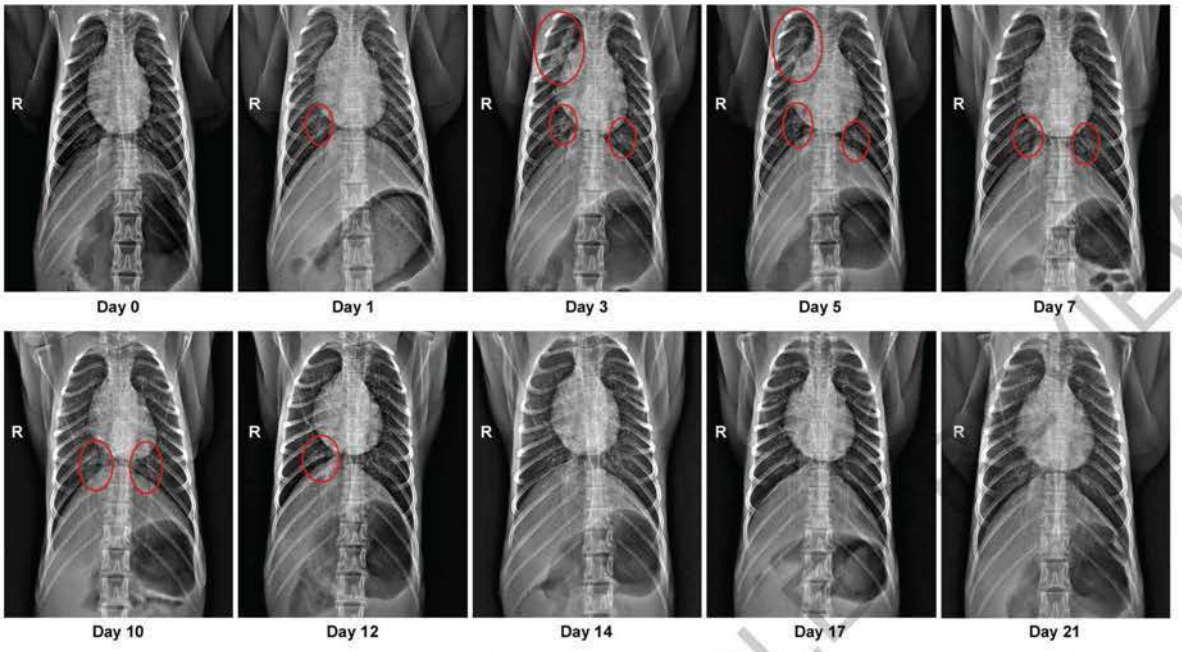
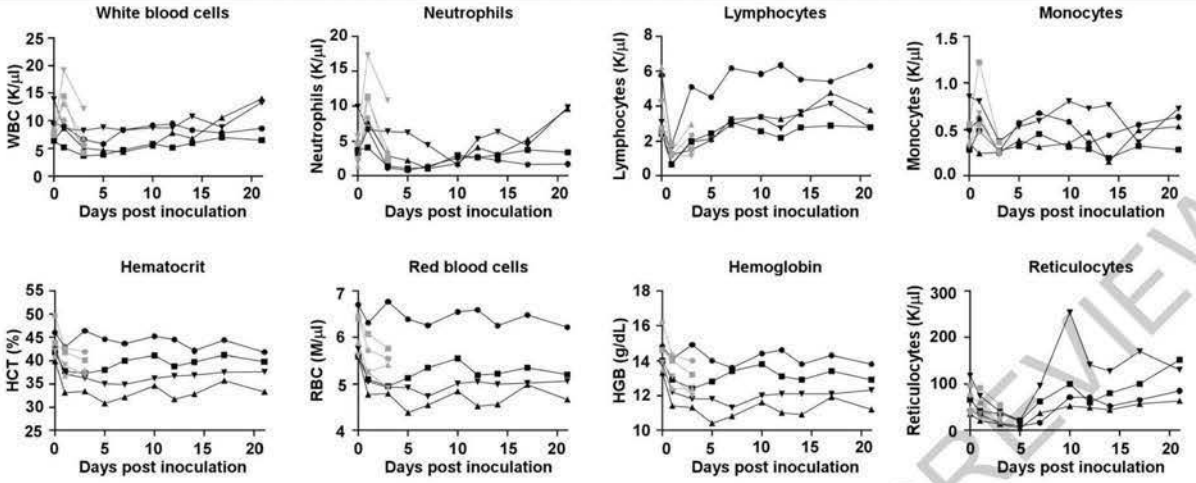
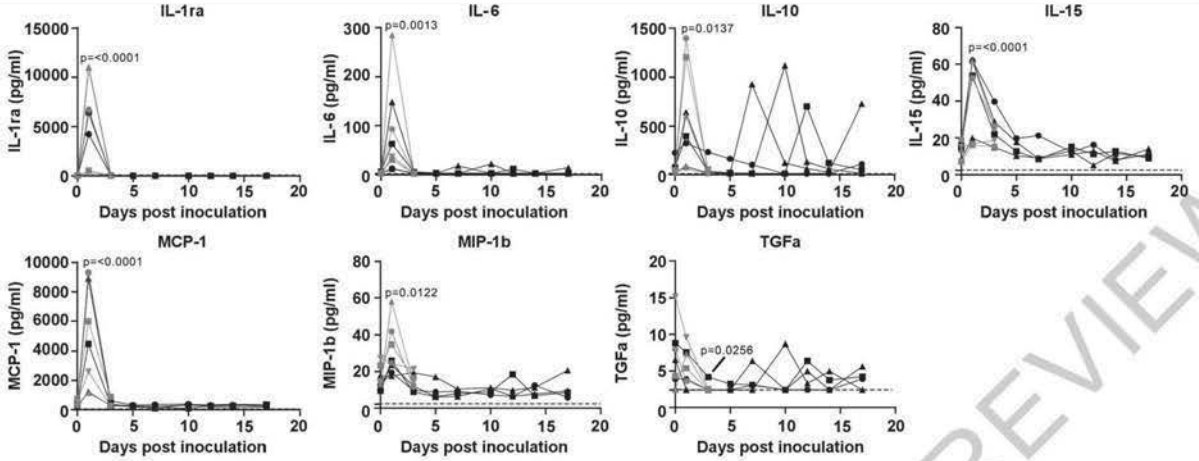


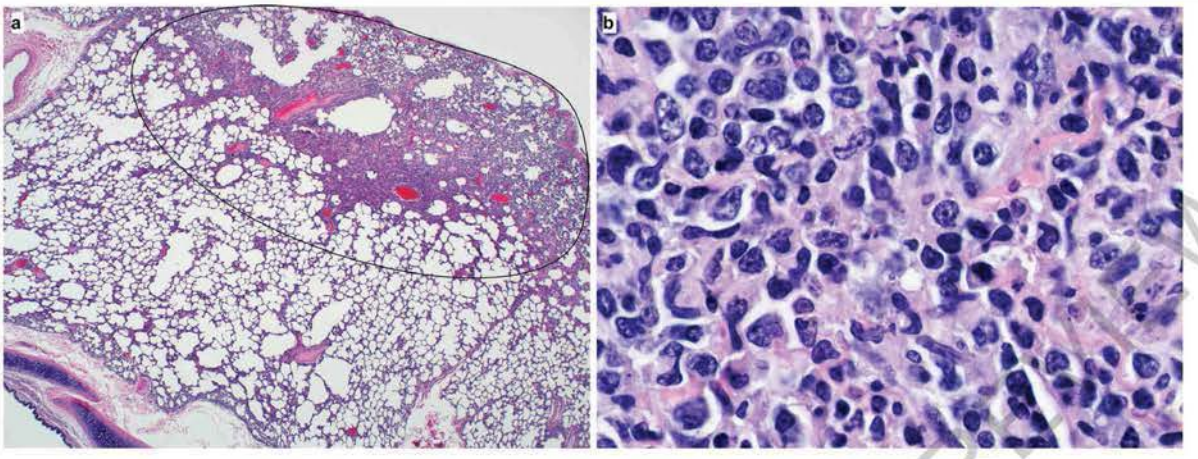
Extended Data Fig. 1 | Pulmonary infiltrates in a rhesus macaque after inoculation. Radiographs show the progression of pulmonary infiltrates throughout the study in a single animal. Of note, this animal is denoted with a black triangle throughout the manuscript. Circles indicate areas of mild to moderate pulmonary infiltrates. A marker 'R' indicates right side of the animal. Three chest radiographs were taken at each timepoint: right-lateral, left-lateral and ventro-dorsal; only the ventro-dorsal radiograph is shown.



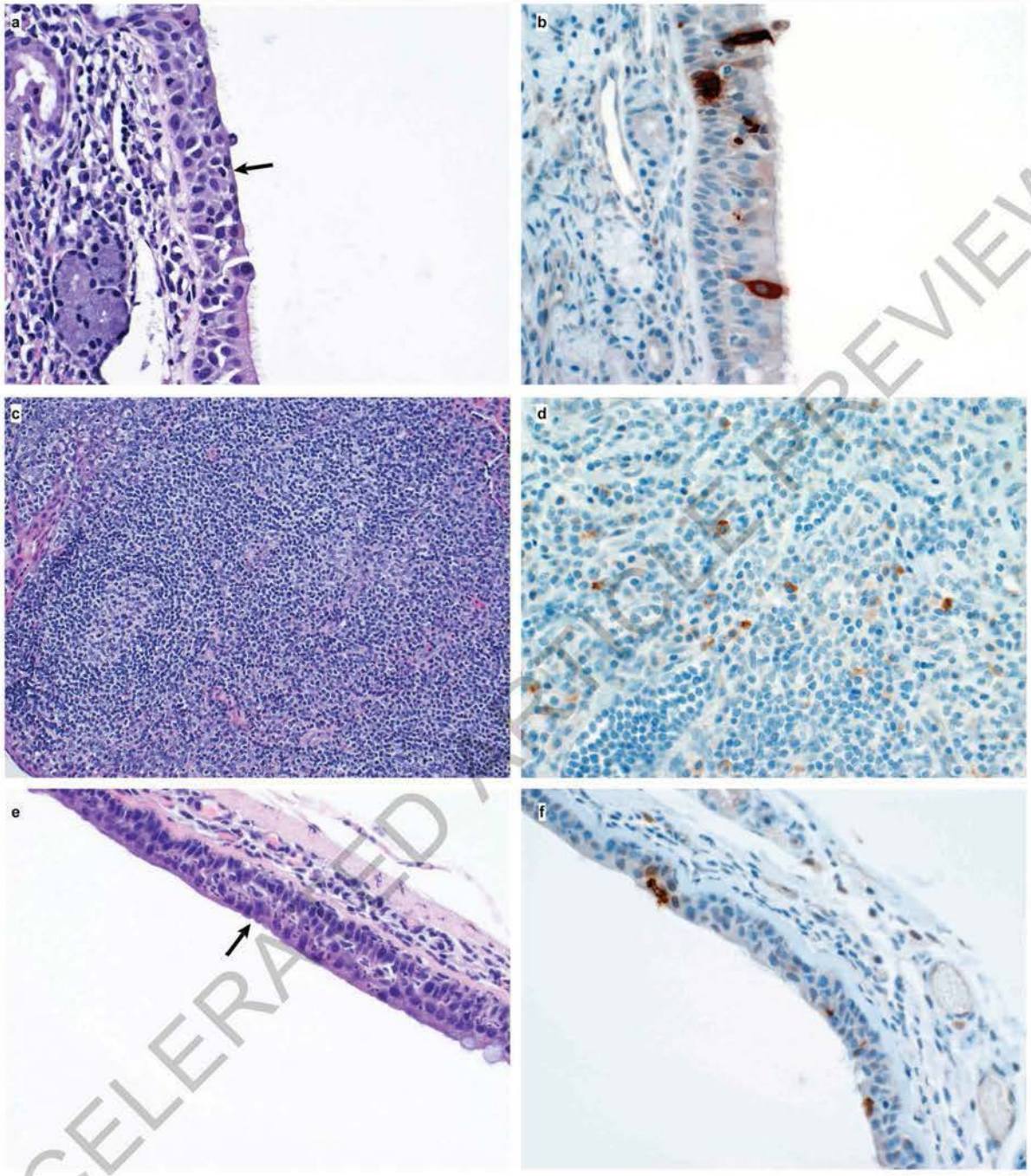
Extended Data Fig. 2 | Hematological changes in rhesus macaques infected with SARS-CoV-2. Identical symbols have been used to denote identical animals throughout the figures in this manuscript. n= 8 animals on 0, 1, and 3 dpi and n=4 animals thereafter.



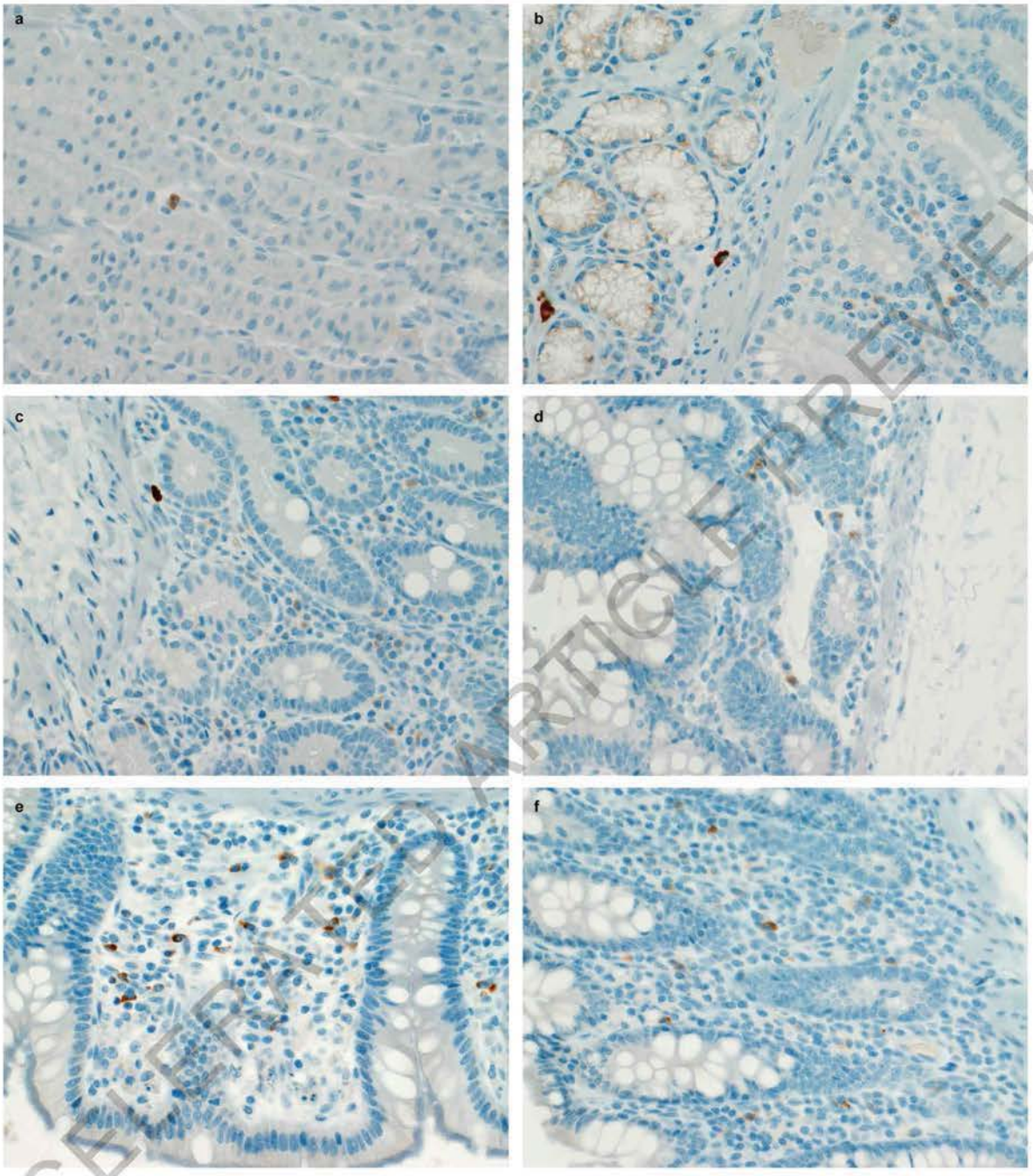
Extended Data Fig. 3 | Cytokine and chemokine levels in serum of rhesus macaques infected with SARS-CoV-2. The levels of 23 cytokines and chemokines were determined in serum at different timepoints after inoculation. Levels are displayed only for those cytokines and chemokines where statistically significant (1-way ANOVA) were observed compared to levels on day of inoculation. Identical symbols have been used to denote identical animals throughout the figures in this manuscript. The lower limit of detection is indicated with a dotted line. Serum samples were analyzed in duplicate from each animal for each timepoint; n= 8 animals on 0, 1, and 3 dpi and n=4 animals thereafter.



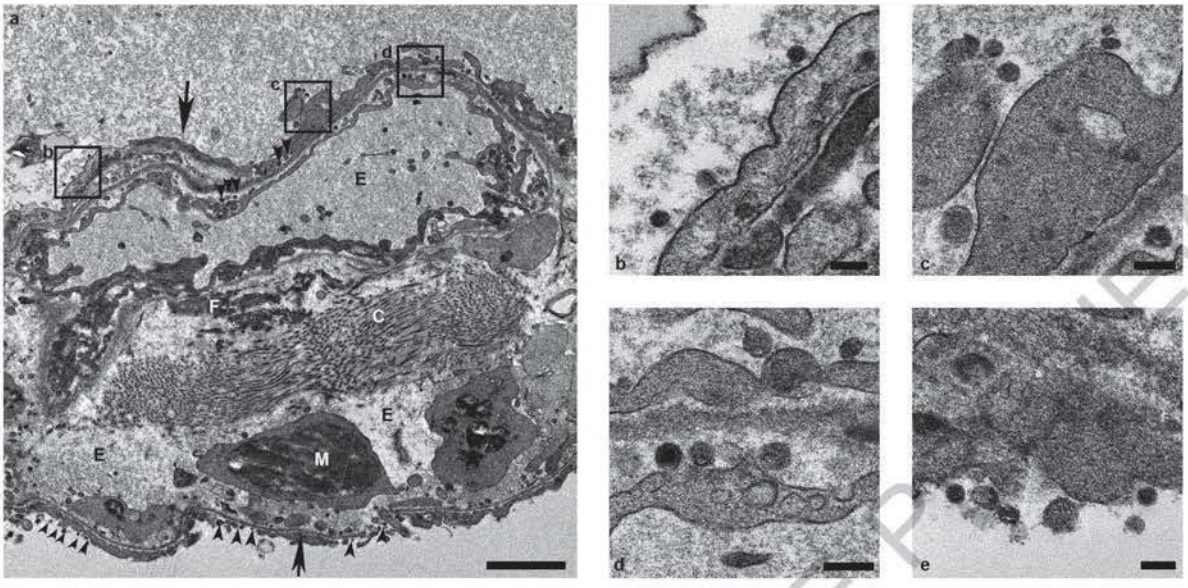
Extended Data Fig. 4 | Histological lesions in lungs of a rhesus macaque infected with SARS-CoV-2. (a) This low magnification figure displays the focal nature of SARS-CoV-2 lesions in the lungs of animals euthanized on 3 dpi. The circle indicates the lung affected by lesion; the remaining lung tissue is healthy. (b) Lymphocytes surround pulmonary vessels. Magnification 500x. Tissue sections were collected from the same anatomical location for each animal; three tissue sections were prepared from each of the 6 lung lobes. In total, 18 lung sections were evaluated for each animal (n=4); representative images are displayed.



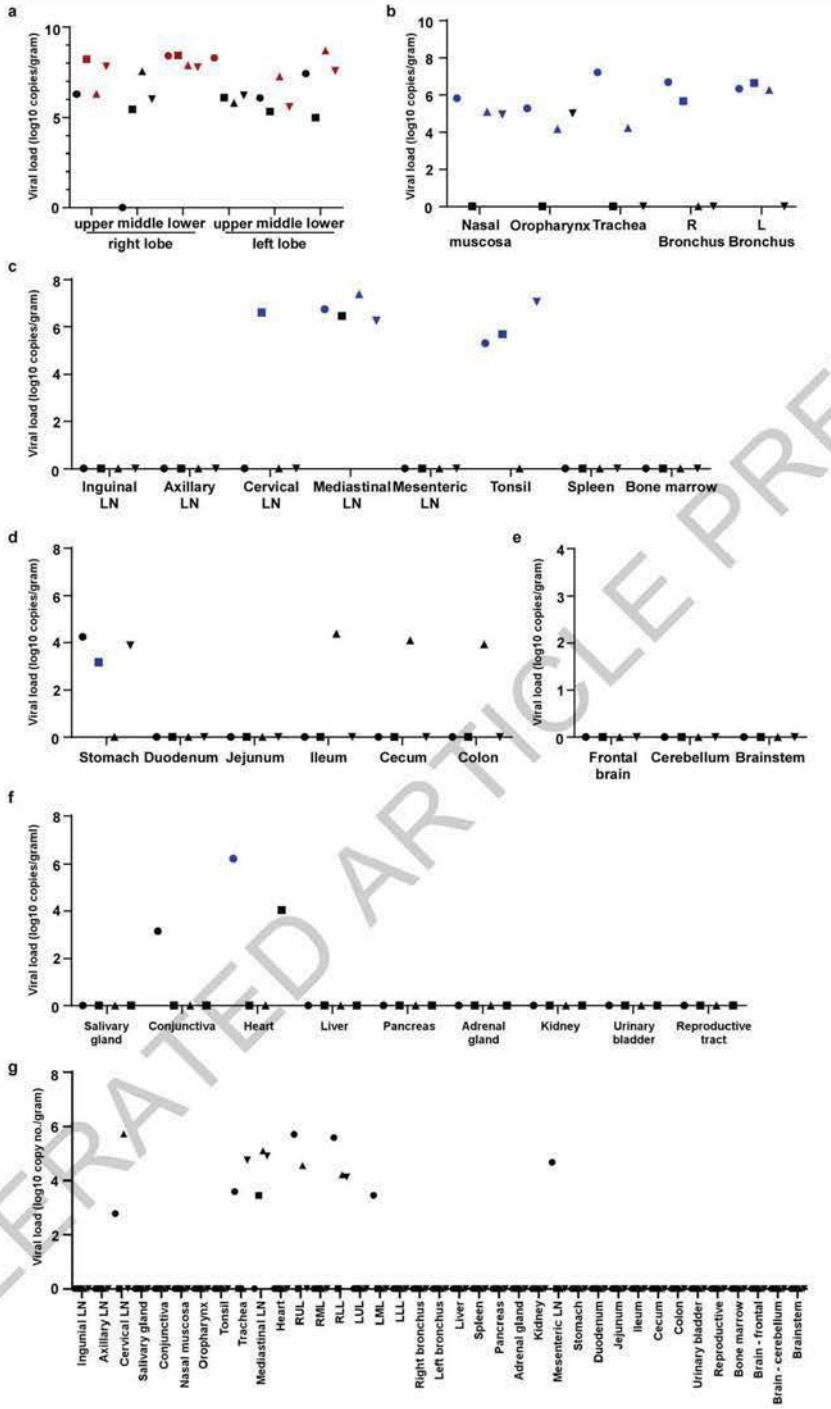
Extended Data Fig. 5 | Histological changes in the respiratory tract of rhesus macaques infected with SARS-CoV-2. (a) Squamous metaplasia of nasal turbinate respiratory epithelium (arrow). Magnification 400x. (b) SARS-CoV-2 antigen is detected by immunohistochemistry in respiratory epithelium of the nasal turbinate. Magnification 400x. (c) Essentially normal tonsil. Magnification 400x. (d) SARS-CoV-2 antigen is detected by immunohistochemistry in tonsillar macrophages. Magnification 400x. (e) Squamous metaplasia of tracheal columnar epithelium (arrow). Magnification 400x. (f) SARS-CoV-2 antigen is detected by immunohistochemistry in tracheal columnar epithelium. Magnification 400x. Tissue sections were collected from the same anatomical location for each animal (n=4) and organ; one tissue section was evaluated of the nasal turbinates of each animal; three tissue sections were evaluated from tonsil and trachea.



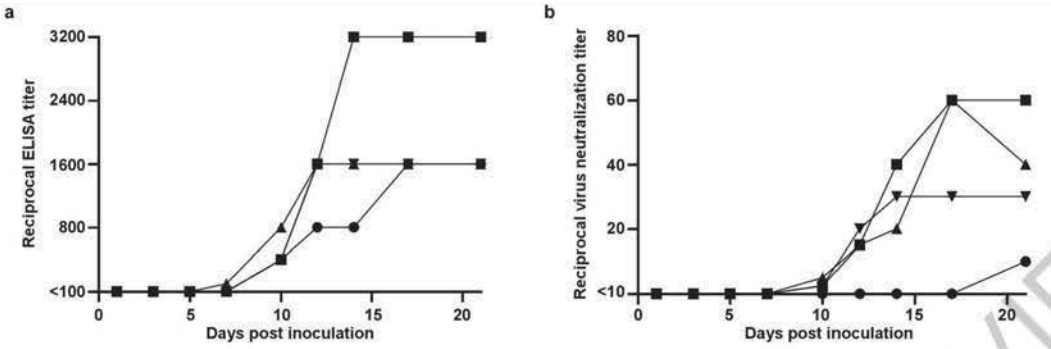
Extended Data Fig. 6 | SARS-CoV-2 antigen in the gastrointestinal tract of a rhesus macaque infected with SARS-CoV-2. Mononuclear cells staining positive for SARS-CoV-2 antigen in the lamina propria of stomach (a), duodenum (b), jejunum (c), ileum (d), cecum (e) and colon (f) of an animal infected with SARS-CoV-2 and euthanized on 3 dpi. Tissue sections were collected from the same anatomical location for each animal (n=4) and organ; three tissue sections were evaluated from each animal and organ.



Extended Data Fig. 7 | Ultrastructural analysis of lungs of rhesus macaques infected with SARS-CoV-2. Lung tissue collected on 3 dpi was analyzed by transmission electron microscopy. The alveolar interstitium is expanded by edema (E), fibrin (F) and mononuclear (M) inflammatory cells (a). Normal collagen fibers (c) and multiple virions (arrowheads) line type I pneumocytes (arrows). Boxes in (a) indicate areas enlarged in (b-d). Scale bar in (a) represents 2µm, scale bars in (b-e) represent 0.2 µm. Three tissue samples were collected from each animal (n=4) and cut into 6 samples for analysis; a minimum of 2 samples were analyzed per animal (n=4).



Extended Data Fig. 8 | Viral loads in tissues collected from rhesus macaques infected with SARS-CoV-2. Eight adult rhesus macaques were inoculated with SARS-CoV-2 isolate nCoV-WA1-2020 and euthanized on 3 (n=4) and 21 (n=4) dpi. Thirty-seven tissues were collected at necropsy and analyzed for the presence of viral RNA by qRT-PCR. Tissues are grouped by lung lobes collected on 3 dpi (a), with red symbols indicating tissues from which virus could be isolated in Vero E6 cells; other tissues from the respiratory tract on 3dpi (b); lymphoid tissues on 3 dpi (c); gastrointestinal tissueson 3 dpi (d); the central nervous system on 3 dpi (e); remaining tissues on 3 dpi (f); and all tissues collected on 21 dpi (g). Blue symbols in b-g indicate that viral mRNA was also detected in these tissues. Identical symbols have been used to denote identical animals throughout the figures in this manuscript. LN: lymph node; RUL: right upper lung lobe; RML: right middle lung lobe; RLL: right lower lung lobe; LUL: left upper lung lobe; LML: left middle lung lobe; LLL: left lower lung lobe; R: right; L: left.



Extended Data Fig. 9 | Antibody response in rhesus macaques infected with SARS-CoV-2. Sera collected after inoculation were tested for the presence of IgG against SARS-CoV-2 spike in ELISA (a) and for the presence of neutralizing antibodies in a microneutralization assay (b). All sera were analyzed in duplicate. Identical symbols have been used to denote identical animals throughout the figures in this manuscript.

Extended Data Table 1 | Clinical signs observed in rhesus macaques inoculated with SARS-CoV-2

Animal	Clinical signs observed 1-6 dpi	Clinical signs observed 7-21 dpi	Observations at necropsy*
RM1	Hunched posture; piloerection; tachypnea; flushed appearance; red eyes; very agitated; reduced appetite; mildly dehydrated. Euthanized 3 dpi.	N/A	Gross lung lesions. Enlarged tonsils and mediastinal lymph nodes. Fluid-filled stomach, small and large intestine.
RM2	Piloerection; dyspnea; reduced appetite. Euthanized 3 dpi.	N/A	Fluid-filled stomach, small and large intestine.
RM3	Piloerection; tachypnea; flushed appearance; reduced appetite; mildly dehydrated. Euthanized 3 dpi.	N/A	Epistaxis. Gross lung lesions. Enlarged mediastinal lymph nodes. Fluid-filled stomach, small and large intestine.
RM4	Hunched posture; piloerection; tachypnea; dyspnea; reduced appetite. Euthanized 3 dpi.	N/A	Gross lung lesions. Foamy exudate from trachea. Enlarged mediastinal lymph nodes. Fluid-filled stomach, small and large intestine.
RM5	Hunched posture; piloerection; tachypnea; dyspnea; reduced appetite.	Tachypnea; dyspnea; reduced appetite; mildly dehydrated. Recovered on 9 dpi.	Gross lung lesions. Enlarged mesenteric lymph nodes.
RM6	Hunched posture; piloerection; tachypnea; dyspnea; reduced appetite; serous nasal discharge.	Piloerection; bradypnea; mildly dehydrated; crusty nasal discharge. Recovered on 10 dpi.	None.
RM7	Hunched posture; piloerection; pale appearance; tachypnea; dyspnea; irregular; labored respirations; anorexia; mildly dehydrated; serous nasal discharge.	Hunched posture; piloerection; pale appearance; tachypnea; dyspnea; reduced appetite; mildly dehydrated; crusty nasal discharge. Recovered on 17 dpi.	None.
RM8	Hunched posture; piloerection; pale appearance; increased, dyspnea; reduced appetite; serous nasal discharge.	Hunched posture; piloerection; pale appearance; increased, dyspnea; nasal discharge; reduced appetite; mildly dehydrated; serous nasal discharge. Recovered on 13 dpi.	Gross lung lesions.

* Incidental observations not related to coronavirus infection were omitted from this table.