

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

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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

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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.

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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, Na₂HPO₄, and KH₂PO₄.

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 BNT162b2 (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 BNT162b2 (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 (CrI: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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