Toxicology Review of COVID-19 Vaccine (BNT162, PF-07302048)
(Final Report)

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File: BLA 125742, original submission

Product: COVID-19 Vaccine (BNT162, PF-07302048)

Reviewer: Nabil Al-Humadi
BLA sections reviewed:
4.2.3.2 Repeated dose toxicity studies
4.2.3.5. Reproductive and Developmental Toxicity

Type and date of submission: Original, August 31st, 2018

Sponsor: BioNTech RNA Pharmaceuticals GmbH, An der Goldgrube 12 Mainz Germany 55131
Proposed indication: Prophylactic immunization against COVID-19 in adults ≥18 years of age
Division name: OVRR/DVRPA

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Précis:

Study number 1:

In this repeat (groups 1 to 5 and 7 animals were dosed by IM on study days 1, 8, and 15 and group 6 animals were dosed on study days 1 and 8) dose toxicology study, rats were assigned to 7 different groups and treated with control or test article (see experimental design). Animals, 18 per sex per group, were treated with a final dose concentration of 0, 10, 30, or 100 [µg/animal]. Animals were euthanized on study days 10 and 17. Except for group 6 ((b) (4) µg/animal [LNP saRNA RBD] test item 5), immune responses were reported in all other treated groups.

Study number 2:

In this repeat (study days 1, 8, and 15) dose toxicology study, rats were assigned to 3 different groups and treated with control or test article (see experimental design). Animals, 15 per sex per group, were treated with a final dose concentration of 30 [µg/animal]. Animals were euthanized on study days 17 and 22. Immune responses were reported in all treated groups.

Study number 3 (Developmental toxicology study):
Animals were randomized and assigned to 4 different groups. Each group consisted of 22 females. Animals were administered 4 doses of saline or test article (30 [µg/animal]) on study day 1 (21 days before mating, M-21) and day 8 (14 days before mating, M-14) and on gestation days 9 and 20. Animals were euthanized according to the following schedule:

F0 Females: Caesarean subset: On GD21.
Littering subset: After weaning of the F1 pups (females that fail to produce a viable litter by GD26 will be euthanized and necropsied).
Unmated Females: After completion of the mating period.
Pups: On PND4 (unselected pups) or on PND21.

Introduction:

Coronavirus infection 2019 (COVID-19) are increasing every day and spreading globally, affecting more and more countries.

The World Health Organization (WHO) characterized the COVID-19 outbreak as pandemic on March 11th, 2020. At the time of writing this report, more than 15 million people around the world were affected and more than 600 thousand people were died. Currently, no approved vaccines or antiviral drugs to prevent or treat SARS-CoV-2 infections or its associated disease COVID-2019 (1).

Significant advantage over more conventional vaccine approaches when using an RNA-based vaccine encoding a viral antigen that is translated to protein by the vaccinated organism to induce a protective immune response. RNA vaccines do not carry the risks associated with infection, unlike live attenuated vaccines. This kind of vaccines may be given to people who cannot be administered live virus (such as pregnant women and immunocompromised persons). The manufacturing of the RNA-based vaccines is via a cell-free *in vitro* transcription process. This method allows an easy and rapid production, and the prospect of producing high numbers of vaccination doses within a shorter time period than achieved with conventional vaccine approaches. In outbreak scenarios, this capability is pivotal to enable the most effective response.

The core innovation of the RNA vaccine is based on *in vivo* delivery of a pharmacologically optimized, antigen-encoding RNA to induce robust neutralizing antibodies and a concomitant T cell response to achieve protective immunization with minimal vaccine doses (2-4).

There are three different RNA platforms under development at BioNTech. These platforms are nonmodified uridine containing mRNA (uRNA, BNT162a), nucleoside modified mRNA (modRNA, BNT162b), and self-amplifying mRNA (saRNA, BNT162c). In more than a dozen non-clinical GLP safety studies, all three RNA platforms have been tested. As for uRNA and modRNA, there is pre-existing clinical safety data. These data have been obtained primarily with

RNAs formulated with (b) (4) which are related, but not identical, to those to be used in this trial.

Generated by BioNTech, the non-clinical toxicity data suggest a favorable safety profile for uRNA and modRNA, as well as saRNA formulated with different nanoparticles for various administration routes, including (b) (4) injection. After (b) (4) dosing, the favorable safety profile is notable because it results in a higher systemic exposure than the planned IM dosing in this trial. The findings from this study were mild and mostly related to the mode-of-action and the RNA-intrinsic stimulation of innate immune sensors. In rodents, the non-clinical safety profile of uRNA and modRNA was predictive for clinical safety.

RNA	Relevant non-clinical safety studies
uRNA	(b) (4)
modRNA	**GLP toxicity studies in mouse:** • 2 studies testing modRNA formulated in LNPs (b) (4) • 2 studies testing modRNA formulated in (b) (4) **Non-GLP safety studies in (b) (4):** • 4 studies testing modRNA formulated in LNPs (b) (4) Route: (b) (4), (other routes in R&D studies) Dose: up to 200 µg/mouse, up to 1.6 mg/kg in (b) (4) monkeys **Overall safety conclusions:** No test-item related findings were noted for (b) (4) formulated modRNA after (b) (4) administration. (b) (4) administration of high doses of LNP formulated modRNA was generally well tolerated. Slight to moderate effects on hematology, clinical parameters (liver enzymes) and on lymphoid organs (spleen, liver) were noted, but were ameliorated after a 2-3 wk recovery period. In studies in (b) (4) monkeys almost no test-item related findings were noted on safety parameters. Slight, but reversible effects on hematology were detected, that were attributed to the mode of action of the encoded proteins.
saRNA	(b) (4)

(b) (4)

Figure 1: BioNTech non-clinical platform experience

Pre-IND meeting was held for this IND on April 06, 2020.

Nonclinical:

Sponsor Question 2:

Does CBER agree that the proposed contents of the nonclinical package, including interim results of the ongoing pivotal GLP rat toxicity study (38166), will be sufficient to support initiation of the planned Phase 1/2 study in the US?

Regarding the ongoing pivotal GLP rat toxicity study (38166), the initial IND will include an interim report with the in-life endpoints (including clinical pathology and partial cytokine results) from the dosing phase. The dosing phase histology, remaining cytokine results, all serology results, and all the recovery phase endpoint results will be submitted as soon as they become available, but no later than 120 days after submission of the IND. Does CBER agree?

FDA Response to Question 2:

We agree that the proposed contents of the nonclinical package, including interim results of the ongoing pivotal GLP rat toxicity study (38166), will be sufficient to support initiation of the planned Phase 1/2 study in the US. We also agree to accept an interim report of the in-life endpoints in the initial IND with the remainder being submitted at a later point in time but no later than 120 days after submission of the IND.

Proposed clinical study:

The clinical study is a multi-site, phase I/II, 2-part, dose-escalation trial investigating the safety and immunogenicity of four prophylactic SARS-CoV-2 RNA vaccines against COVID-2019 using different dosing regimens in healthy adults.

In this study four different vaccines (BNT162a1, BNT162b1, BNT162b2, and BNT162c2) will be tested. Two parts will be included in this study:

Part A

A dose-finding part with four dose cohorts (treatment groups) for each vaccine and one pre-defined and one optional dose level for a de-escalation approach. A dose-escalation design will be followed in the first part of the trial (part A). Subjects in this trial (first-in-human [FIH] immunization) will be immunized using a sentinel dosing/subject staggering (EMA 2017 guidance “Strategies to Identify and Mitigate Risks for First-in-Human and Early Clinical Trials with Investigational Medicinal Products”). The table below shows the FIH starting dose and the planned escalation/de-escalation doses:

Vaccine	mRNA type	Vaccine encoded antigen	Vaccine IM dosing regimen	Part A - Dose Groups & Dose (µg) (12 subjects per cohort)				Part B - Optional Expansion Cohorts
				1 Starting dose	2	3 De-escalation dose	4	
BNT162a 1	uRNA	RBD of the SARS-CoV-2 S protein	Prime: Day 1 Boost: Day 22	(b) (4)				
BNT162b 1	modRNA	RBD of the S protein	Prime: Day 1 Boost: Day 22	1B 10 µg	2B 30 µg	(b) (4)	4B 100 µg	As above
BNT162b 2	modRNA	A modified version of the S protein	Prime: Day 1 Boost: Day 22	1C 10 µg	2C 30 µg	3C 1 µg	4C 100 µg	As above
BNT162c 2	saRNA	A modified version of the S protein	Prime only: Day 1	(b) (4)				As above

IM = intramuscular; RBD = Receptor Binding Domain; S protein = SARS-CoV-2 Spike protein

Figure 2: Summary of vaccine dose regimens in the clinical study

PART A : Dose Cohort Scheme for uRNA (BNT162a1) and saRNA (BNT162c1)

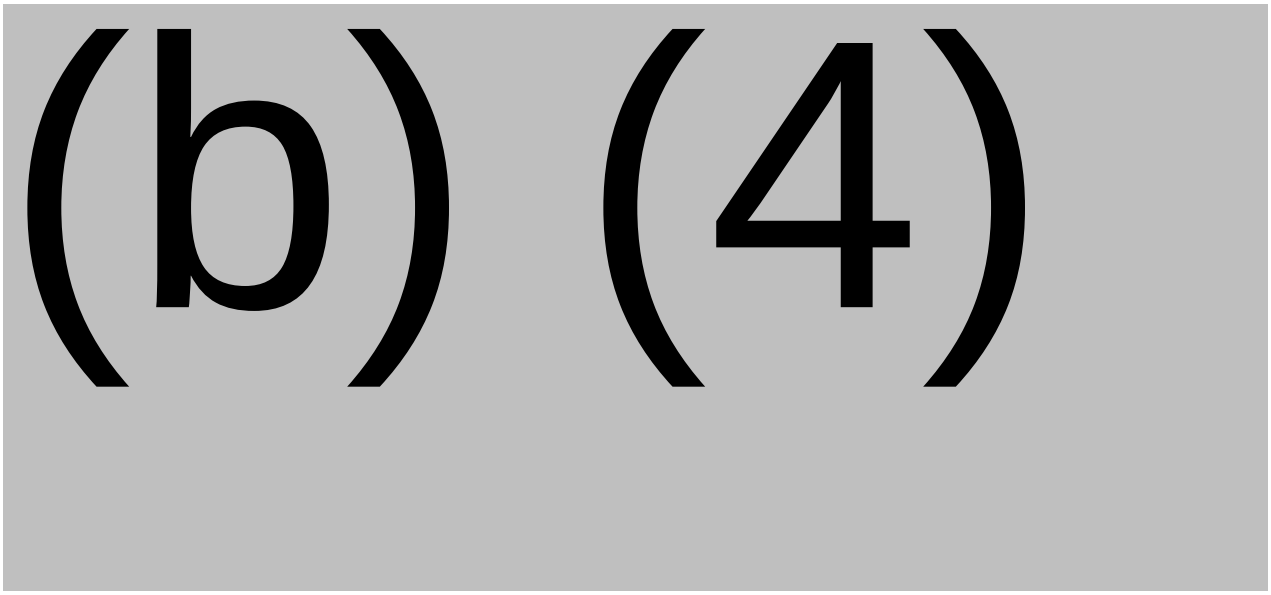


Figure 3: Part A; Dose cohort scheme for uRNA (BNT162a1) and saRNA (BNT162c1)

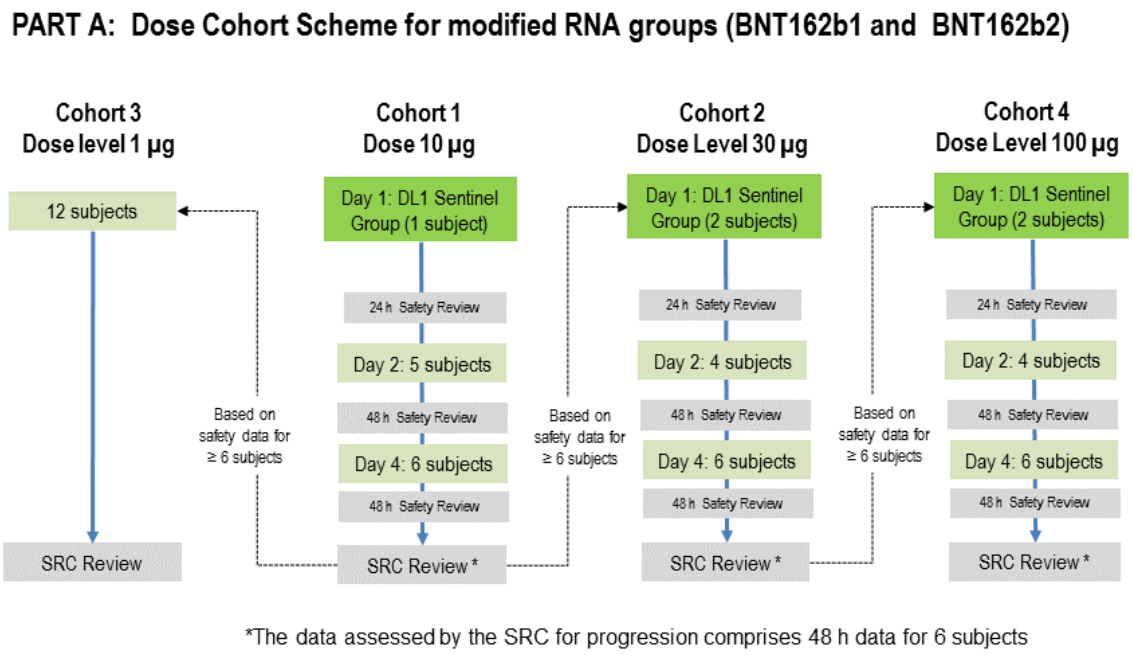


Figure 4: Part A; Dose cohort scheme for modified RNA groups (BNT162b1 and BNT162b2)

DL = Dose level; SRC = Safety Review Committee.
Figure of graphical depiction of the dose-finding process in part A

Part B
Dedicated to recruit expansion cohorts with dose levels which are selected from data generated in part A. Using a P/B regimen, the vaccines BNT162a1, BNT162b1, and BNT162b2 will be administered. For the vaccine BNT162c2, SD regimen will be used. After evaluation of aggregate data from part A, details of part B will be defined using a protocol amendment. Based on analysis of both immunogenicity and safety data gathered in part A, progression to part B will be decided. Immunogenicity and safety will be thoroughly assessed to select the vaccine and the dose(s) to be further evaluated in part B.

Safety data to be evaluated includes the package used by the SRC to assess individual dose levels. Immunogenicity of all doses will be assessed. In the protocol amendment, a summary of relevant safety and tolerability data collected in part A will be included. Also, the protocol amendment will include part B specific inclusion/exclusion criteria, objectives/endpoints, a description of the planned statistical analyses, and descriptions of any added trial assessments and procedures.

The design of part B will be a randomized, placebo-controlled in the likely target population (e.g., high risk populations such as elderly and/or immunocompromised populations). Part B may employ a surrogate marker as a measure of vaccine efficacy.

Studies reviewed for this BLA:

1- Repeat-dose toxicity study of three LNP-formulated RNA platforms encoding for viral proteins by repeated intramuscular administration to Wistar Han rats. Study number: 38166 (submitted in amendment 0).

2- 17-day intramuscular toxicity study of BNT162B2 (V9) and BNT162B3C In Wistar Han rats with a 3-week recovery. Study number: 20GR142 (submitted in amendment 32).
3- A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by the Intramuscular Administration in the Wistar Rat. Study number: 20256434 (submitted in amendment 141).

Studies not reviewed in all amendments:
None.

Toxicology Study Review

Study number 1:

Title and study number: Repeat-dose toxicity study of three LNP-formulated RNA platforms encoding for viral proteins by repeated intramuscular administration to Wistar Han rats. Study number: 38166.

Performing laboratory: (b) (4)

Study initiation date: March 17, 2020
Final report date: July 1, 2020

Test article batch/lot:

Test Article		Batch Number	Stability
Buffer (	(b) (4))	(b) (4)	Not reported
(b) (4)	(BNT162a - 1)	(b) (4)	Not reported
(b) (4)	(BNT162b - 1)	(b) (4)	Not reported
RBP020.1"	(BNT162b - 2)	CoVVAC/160320	Not reported
(b) (4)	(BNT162c - 1)	(b) (4)	Not reported

Animal species and strain: Rat/Wistar/(b) (4):WI(Han)
Breeder/supplier: (b) (4)

Number of animal per group and sex: 15/sex/group
Age: Approximately 10-14 weeks at 1st dosing
Body weight range:
Males: 252.8g-343.9g
Females: 188.3g-267.3g
Route and site of administration: Intramuscular (IM)
Volume of injection: 0.5 mL

Frequency of administration and study duration:
For groups 1 to 5 and 7:
On test days 1, 8 and 15; in total 3 administration days at one-week intervals per animal.
For group 6:
On test days 1 and 8; in total 2 administration days at one-week interval per animal.

Dose: See study design

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. At the time of submitting this study, stability studies with the first clinical trial material batch have just been started. Up to now no results are available. Stability data will be included in any upcoming amendment. The table below shows the protocol of stability study I for CTM drug substance batches:

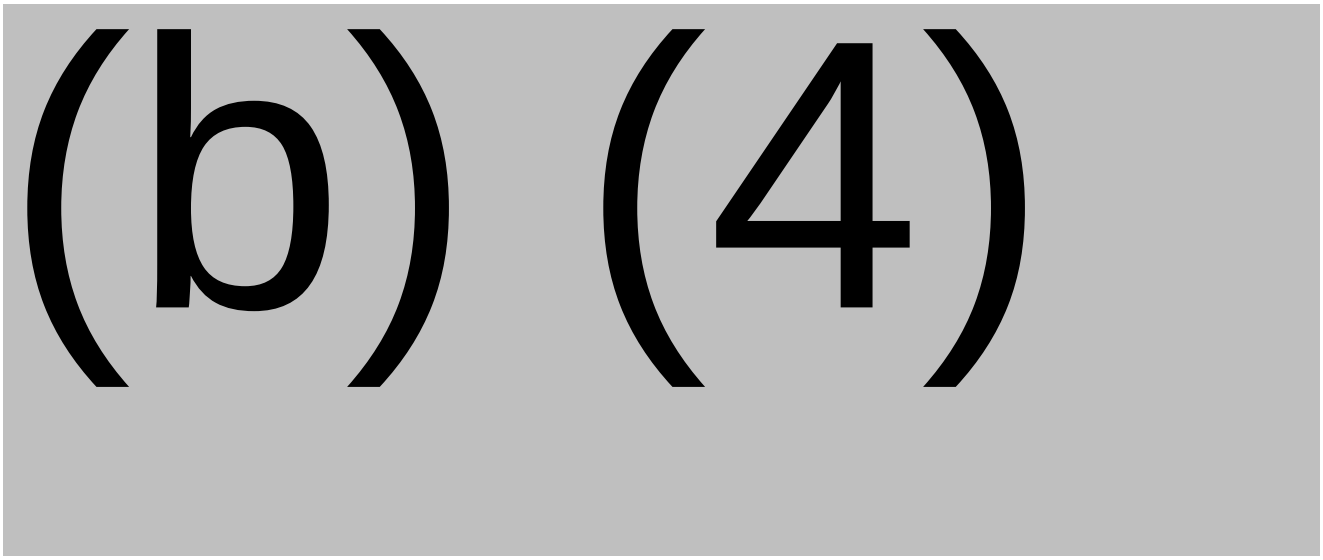


Table 1: Protocol of stability study I for CTM drug substance batches at different storage conditions

Means of administration: Intramuscular (IM)
Report status: Interim report

Experimental design:
Animals were randomized and assigned to 7 different groups. Each group consisted of 18/sex/group. Groups 1 to 5 and 7 animals were dosed by IM on study days 1, 8, and 15. Groups 6 animals were dosed by IM on study days 1 and 8. The details of the study design are listed in the following table:

Group	Dose level [µg/animal] (Test item / Control)	No. and sex of animals MS + RP + SA	Rat number		
			MS	RP	SA
1	0 (Buffer) Control	10 + 5 + 3 m 10 + 5 + 3 f	1-10 16-25	11-15 26-30	211-213 214-216
2	(b) (4) (LNP uRNA RBD) Test item 1	10 + 5 + 3 m 10 + 5 + 3 f	31-40 46-55	41-45 56-60	217-219 220-222

Group	Dose level [µg/animal] (Test item / Control)	No. and sex of animals MS + RP + SA	Rat number		
			MS	RP	SA
3	(b) (4) (LNP uRNA RBD) Test item 1	10 + 5 + 3 m 10 + 5 + 3 f	61-70 76-85	71-75 86-90	223-225 226-228
4	(b) (4) (LNP modRNA RBD) Test item 3	10 + 5 + 3 m 10 + 5 + 3 f	91-100 106-115	101-105 116-120	229-231 232-234
5	(b) (4) (LNP modRNA RBD) Test item 3	10 + 5 + 3 m 10 + 5 + 3 f	121-130 136-145	131-135 146-150	235-237 238-240
6	(b) (4) (LNP saRNA RBD) Test item 5	10 + 5 + 3 m 10 + 5 + 3 f	151-160 166-175	161-165 176-180	241-243 244-246
7	100 (LNP modRNA Sp2) Test item 4	10 + 5 + 3 m 10 + 5 + 3 f	181-190 196-205	191-195 206-210	247-249 250-252
Erroneously treated animals#:	(b) (4) (LNP uRNA RBD) Test item 1	0 + 0 + 3 m	-	-	253-255

m: male, f: female, MS: Main study, RP: Recovery period, SA: Satellite animals for cytokine analysis (except last group). #: Due to shortly planned dose reduction of group 3, three animals had already been dosed as originally planned with (b) (4)/animal. These three animals were replaced by 3 spare animals in group 3. The three erroneously treated animals were maintained for at least 48 hours as a non-GLP group with observations reported informally to the sponsor (body weight (test day 1 and 24 and 48 hours post injection), body temperature (24 and 48 hours post injection) and local tolerance (24 and 48 hours post injection)).

Table 2: Experimental design

Methods:
Randomization procedure: Yes
Statistical analysis plan: Yes.

The following parameters were evaluated: Clinical observations (twice daily), local tolerance [Draize scoring] (4, 24, and 48 hours after each injection), body weights (prior to injection on study days 1, 8, and 15, after treatment on study days 2, 9, and 16, and at necropsy on study days 10 or 17), food consumption (weekly), ophthalmology (before first dosing and at the end of the dosing period), body temperature (4 and 24 hours post injection on study days 1, 8, and 15), cytokines (study days 1, 8, 10, 15, and 17), clinical chemistry, hematology, coagulation, and

acute phase proteins (study days 4, 10, and 17), urinalysis (study days 10 and 17), serology (day 10 [BNT162c1] or at day 17 after first immunization [BNT162a1, BNT162b1, and BNT162b2]). Postmortem evaluations were performed on study days 10 (groups 6 and 7) and 17 (groups 1 to 5).

Parameters	Frequency of Testing
Cageside observation[1]	Twice daily
Clinical observations [2]	Twice daily
Body weight	Prior to injection on study days 1, 8, and 15, after treatment on study days 2, 9, and 16, and at necropsy on study days 10 or 17
Food consumption	Weekly
Body temperature	4 and 24 hours post injection on study days 1, 8, and 15
Ophthalmologic exam	Before first dosing and at the end of the dosing period
Clinical chemistry*	Study days 4, 10, and 17
Hematology*	Study days 4, 10, and 17
Coagulation*	Study days 4, 10, and 17
Local tolerance [Draize scoring]	4, 24, and 48 hours after each injection
Serology	Day 10 (BNT162c1) or at day 17 after first immunization (BNT162a1, BNT162b1, and BNT162b2)
Cytokines	Study days 1, 8, 10, 15, and 17
Urinalysis	Study days 10 and 17
Postmortem study evaluations	Study days 10 (groups 6 and 7) and 17 (groups 1 to 5)

* Site collection of blood samples were retrobulbar venous plexus.

Day of sampling	**Animals**	**Parameters**
Test day 4:	The first 5 main study animals per sex and group and all recovery animals.	Hematology Clinical chemistry Acute phase proteins
At main study termination (on the day of dissection, i.e. on test days 10 or 17):	All main study animals	Hematology Coagulation Clinical chemistry Acute phase proteins

Table 3: Blood sampling schedule for laboratory examinations

[1] Cageside observations include mortality, morbidity, general health and signs of toxicity.
[2] Clinical observations include evaluation of skin and fur, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior.

Parameter	Matrix	Total amount of sample	Aliquots prepared	Storage temperature	(b) (4) Kit
α1-acid glycoprotein	Serum	150 µL	2 x 75 µL	-20°C ± 10%	Rat Alpha 1 Acid Glycoprotein / AGP (b) (4)
α2 macroglobulin	Serum	150 µL	2 x 75 µL	-20°C ± 10%	Rat alpha 2 Macroglobulin (b) (4)

Table 4: Acute phase proteins

Cytokines	Matrix	Total amount of sample	Aliquots prepared	Storage temperature	Method
IFN-γ TNF-α IL-1-β IL-6 IL-10	Serum	150 µL	2 x 75 µL	–20°C ± 10%	(b) (4)

Table 5: Cytokine analysis

Postmortem procedures:
Table of weighed organs

Adrenal gland (2)	Ovary (2)
Brain	Pituitary gland
Epididymis (2)	Prostate
Heart	Spleen
Kidney (2)	Testicle (2)
Liver	Thymus
Lungs	Thyroid (1) (including parathyroids)
Lymph nodes (cervical (1), mesenteric (1))	

Table 6: Weighed organs

Results:
No test article-related mortality was reported.

Clinical chemistry and hematology:

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≥ 1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Alanine aminotransferase (ALT or SGPT) SD4 F ↓ = 0.6 G7	Aspartate aminotransferase (AST or SGOT)
B) HEPATOBILIARY		Total bilirubin Alkaline phosphatase (ALP)
ACUTE PHASE REACTANTS		Fibrinogen (also under coagulation)
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Fasting triglycerides SD4 M (b) (4) SD4 M SD4 M SD4 M SD4 M ↓ = 0.3 G7 SD4 F (b) (4) SD4 F SD4 F SD4 F SD4 F ↓ = 0.3 G7 SD17 F (b) (4) Total Cholesterol SD17 M (b) (4) SD17 M Creatine kinase (CK) SD4 M (b) (4) Gamma-GT SD4 M (b) (4) SD4 M SD4 M SD4 M SD4 M SD4 M ↑ = 3.4 G7 SD17 M (b) (4) SD17 M SD17 M SD17 M SD17 M ↑ = 3.0 G7	Albumin (A) Total protein Carbon dioxide Globulin A/G ratio

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≥ 1.5)**)**	NOT OF NOTE
	SD4 F (b) (4) SD4 F SD4 F SD4 F SD4 F SD4 F ↑ = 4.6 G7 Lactate dehydrogenase (LDH) SD4 F (b) (4)	

Table 7: Serum chemistry results

Clinical chemistry results showed a decrease in ALT levels in group 7 females at study day 4. Triglyceride levels were decreased in groups (b) (4) 7 males at study day 4. Triglyceride levels were decreased in groups (b) (4) 7 females at study day 4. Triglyceride levels were (b) (4) in group (b) (4) females at study day 17. Cholesterol levels were (b) (4) in groups (b) (4) males at study day 17. Creatine kinase levels were (b) (4) in group (b) (4) males at study day 4. Gamma-GT levels were increased in groups (b) (4) 7 males at study day 4. Gamma-GT levels were increased in groups (b) (4) 7 males at study day 17. Gamma-GT levels were increased in groups (b) (4) 7 females at study day 4. LDH levels were (b) (4) in group (b) (4) females at study day 4.

Figure 5: Gamma-glutamyltransferase plasma activity in male rats mean values per group and standard deviation. TD = Treatment day.

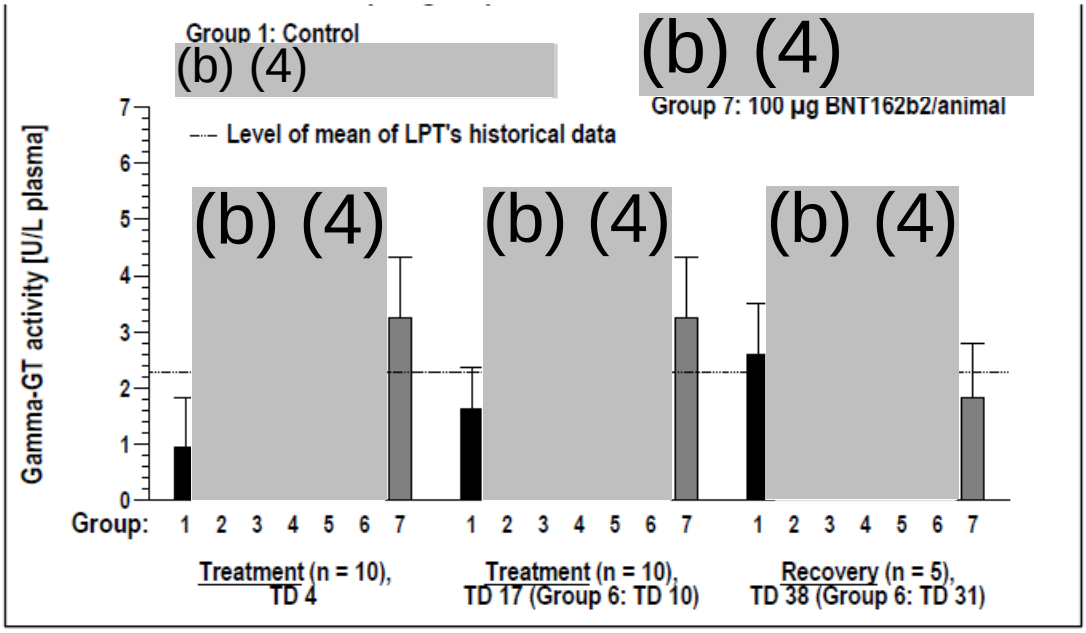


Figure 6: Gamma-glutamyltransferase plasma activity in female rats mean values per group and standard deviation. TD = Treatment day.

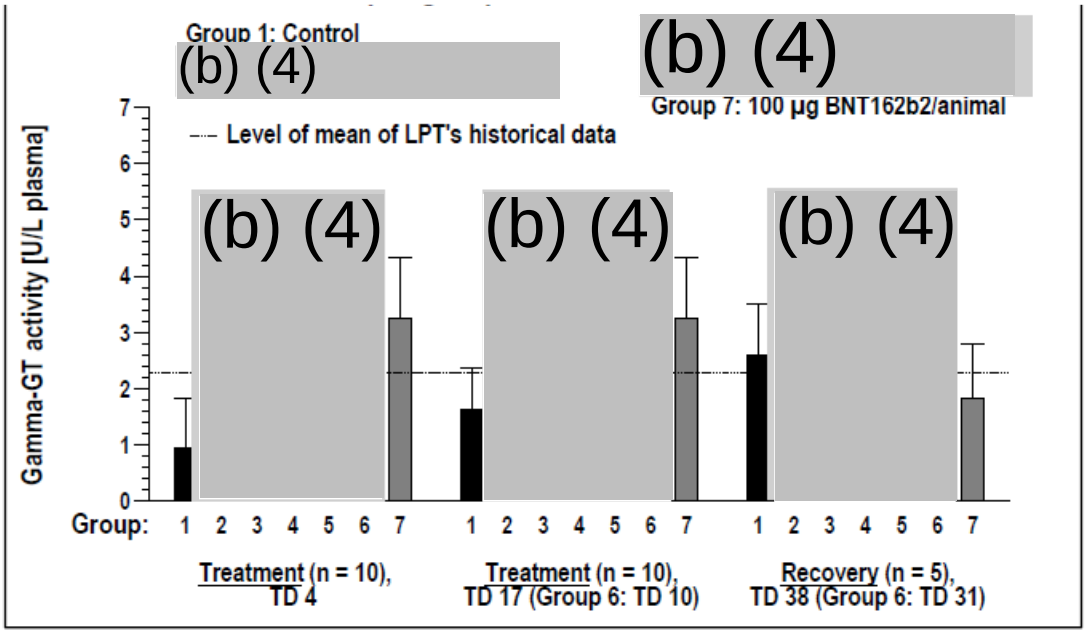
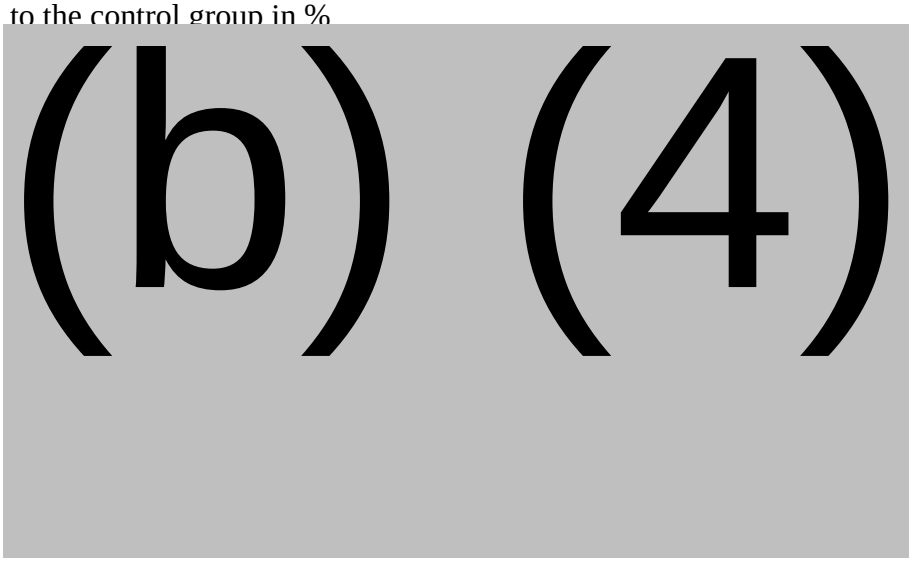


Figure 7: Test article-related changes in plasma activity of gamma-glutamyltransferase compared to the control group in %



In all test article-treated groups, an increase in albumin plasma levels and a decrease in globulin plasma levels, resulting in an altered albumin/globulin ratio, were reported. These changes are consistent with an acute phase response in albumin and globulin where albumin goes down and globulin goes up with inflammation, and the albumin/globulin ratio decreases. The following table lists the statistically significant changes reported in albumin and globulin levels and the alb./glob. ratio.

Statistically significant differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group						
Parameter	**Group**	**Test item**	**Dose [µg/animal]**	**Sex**	**Test day**	**Change [%]**
Albumin	2	BNT162a1	(b) (4)	m	4	(b) (4)
					17	
				f	4	
					17	
	3	BNT162a1		m	4	
					17	
				f	4	
					17	
	4	BNT162b1		m	4	
					17	
				f	4	
					17	
	5	BNT162b1		m	4	
					17	

Statistically significant differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group						
Parameter	**Group**	**Test item**	**Dose [µg/animal]**	**Sex**	**Test day**	**Change [%]**
				f	4	(b) (4)
					17	
	6	BNT162c1	(b) (4)	m	4	
				f	4	
	7	BNT162b2	100	m	4	-9.1**
					17	-5.9**
				f	4	-12.6**
					17	-11.0**
Globulin	2	BNT162a1	(b) (4)	m	4	(b) (4)
					17	
				f	17	
	4	BNT162b1		m	4	
					17	
				m	17	
				f	17	
	5	BNT162b1		m	4	
					17	
				f	17	
	6	BNT162c1		m	4	
	7	BNT162b2	100	m	4	+7.3*
					17	+23.1**
				f	17	+17.7**
Albumin/Globulin Ratio	2	BNT162a1	(b) (4)	m	4	(b) (4)
					17	
				f	4	
					17	
	3	BNT162a1		m	4	
					17	
	4	BNT162b1		m	4	
					17	
				f	4	
					17	
	5	BNT162b1		m	4	

Statistically significant differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group						
Parameter	**Group**	**Test item**	**Dose [µg/animal]**	**Sex**	**Test day**	**Change [%]**
					17	(b) (4)
				f	4	
					17	
	6	BNT162c1	(b) (4)	m	4	
				f	4	
	7	BNT162b2	100	m	4	-15.1**
					17	-23.6**
				f	4	-15.7**
					17	-24.4**

m = Male
f = Female

*/** Statistically significant at p = 0.01 / p = 0.05 (based on numerical data, not on percent difference).

Table 8: Differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.53, ie, ≥1.6 or ≤ 1.6	Not of NOTE
Red blood cells	Reticulocytes SD4 M (b) (4) SD4 M SD4 M SD4 M SD4 M ↓ = 0.3 G7 SD4 F (b) (4) SD4 F SD4 F SD4 F SD4 F ↓ = 0.5 G7	Hematocrit (Hct) Hemoglobin Conc. (Hb) Mean Corp. Hb. (MCH) Mean Corp. Hb. Conc. (MCHC), Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
White blood cells	Monocyte count: SD4 F (b) (4) SD4 F SD17 F (b) (4) SD17 F SD17 F	Macrophage Leukocytes

[3] With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.53, ie, ≥1.6 or ≤ 1.6	Not of NOTE
	SD17 F (b) (4) SD17 F ↑ = 1.6 G7 Lymphocyte count SD17 M (b) (4) SD17 M SD17 M ↓ = 0.5 G7 Neutrophil count SD4 M (b) (4) SD4 M SD17 M (b) (4) SD17 M SD17 M SD17 M SD17 M ↑ = 3.2 G7 SD4 F (b) (4) SD4 F SD4 F SD4 F ↑ = 2.3 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F SD17 F ↑ = 7.8 G7 Eosinophils count SD4 M (b) (4) SD17 M (b) (4) SD17 M SD17 M SD17 M ↑ = 2.2 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F ↑ = 6.1 G7 Basophils SD4 M (b) (4) SD4 M SD4 M SD4 M ↑ = 2.5 G7 SD17 M (b) (4) SD17 M SD17 M SD17 M SD17 M ↑ = 2.5 G7 SD4 F (b) (4)	

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.53, ie, ≥1.6 or ≤ 1.6	Not of NOTE
	SD4 F (b) (4) SD4 F ↑ = 1.7 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F SD17 F ↑ = 2.1 G7 White Blood Cells (WBC) SD17 M (b) (4) SD17 M SD17 M SD17 M SD17 M ↑ = 2.2 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F SD17 F ↑ = 2.1 G7 Large Unstained Cells (LUC) SD4 M (b) (4) SD4 M SD4 M SD4 M SD4 M ↑ = 2.8 G7 SD17 M (b) (4) SD17 M SD17 M SD17 M SD17 M ↑ = 3.4 G7 SD4 F (b) (4) SD4 F SD4 F SD4 F SD4 F ↑ = 4.2 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F SD17 F SD17 F ↑ = 4.2 G7	
Clotting potential	Platelet count SD17 F (b) (4) Fibrinogen SD17 M (b) (4) SD17 M SD17 M	Activated partial-thromboplastin time clotting time Prothrombin time

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.53, ie, ≥1.6 or ≤ 1.6	Not of NOTE
	SD17 M (b) (4) SD17 M ↑ = 3.1 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F SD17 F ↑ = 2.6 G7 PCT % SD17 M (b) (4) SD17 M SD17 M ↓ = 0.6 G7 SD17 F (b) (4) SD17 F SD17 F SD17 F ↓ = 0.6 G7	
Others		Bone marrow cytology

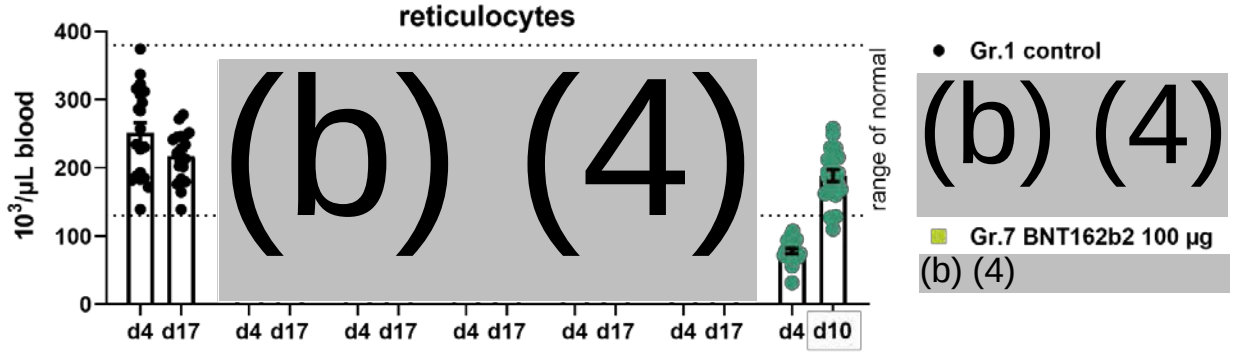
Table 9: Hematological results

Day: 10 Relative to Start Date

Sex: Male		Haematological Parameters						
		HGB (mmol/L) [a]	RBC (x10E6/µL) [a]	WBC (x10E3/µL) [a]	Reti (%) [a]	Reti (x10E3/µL) [a]	PLT (x10E3/µL) [a]	HCT (%) [a]
Group 6: (b) (4) animal T. item 5	Mean SD N	(b) (4)						
		-	-	-	-	-	-	-

Hematology results showed decrease in reticulocyte levels in groups (b) (4) 7 males at study day 4. Reticulocyte levels were decreased in groups (b) (4) 7 females at study day 4. Reticulocytes levels were decreased after the 1st dose but recovered by the end of in-life of the toxicity study.

Figure 8: Reticulocyte's levels



Monocyte levels were (b) (4) in groups (b) (4) females at study day 4. Monocyte levels were increased in groups (b) (4) 7 females at study day 17. Lymphocyte levels were decreased in groups (b) (4) 7 males at study day 17. Neutrophil levels were (b) (4) in group (b) (4) males at study day 4. Neutrophil levels were (b) (4) in group (b) (4) males at study day 4. Neutrophil levels were increased in groups (b) (4) 7 males and females at study day 17. Neutrophil levels were increased in groups (b) (4) 7 females at study day 4. Eosinophil levels were (b) (4) in group (b) (4) males at study day 4. Eosinophil levels were (b) (4) in groups (b) (4) males at study day 17. Eosinophil levels were increased in groups (b) (4) 7 males at study day 17. Eosinophil levels were increased in groups (b) (4) 7 females at study day 17. Basophil levels were increased in groups (b) (4) 7 males at study day 4. Basophil levels were increased in groups (b) (4) 7 males at study day 17. Basophil levels were increased in groups (b) (4) 7 females at study day 4. Basophil levels were increased in groups (b) (4) 7 females at study day 17. WBC levels were increased in groups (b) (4) 7 males and females at study day 17. LUC levels were increased in groups (b) (4) 7 males and females at study day 4. LUC levels were increased in groups (b) (4) 7 males and females at study day 17.

Platelet count were (b) (4) in group (b) (4) females at study day 17. Fibrinogen levels were increased in groups (b) (4) 7 males and females at study day 17. PCT% levels were decreased in groups (b) (4) 7 males at study day 17. PCT% levels were decreased in groups (b) (4) 7 females at study day 17.

Groups 2 and 3:
(b) (4)

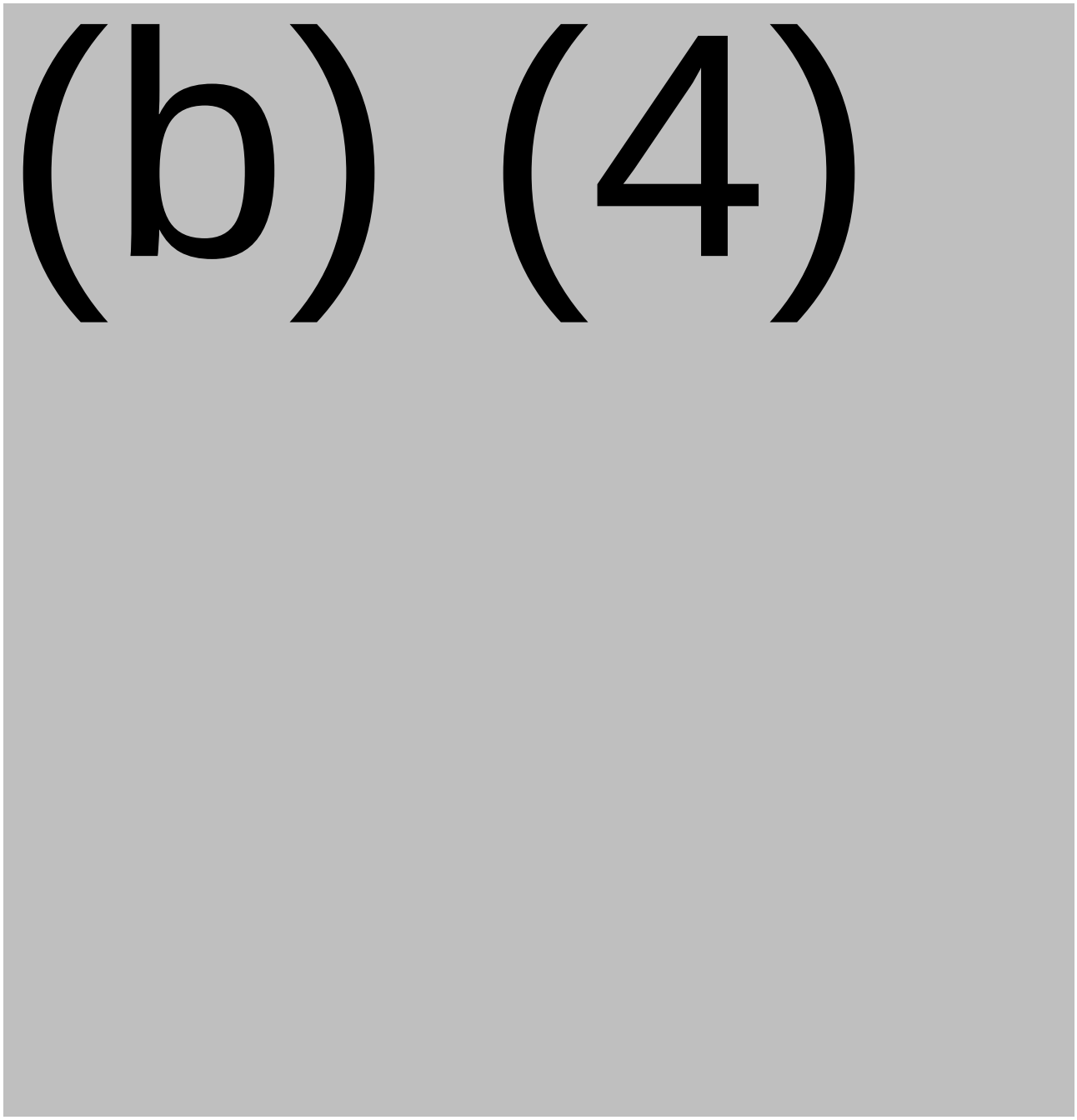
(b) (4)

Groups 4 and 5:
(b) (4)

(b) (4)

BNT162c1 - Group 6

(b) (4)



BNT162b2 - Group 7

Test article-related changes included decreases in the absolute and relative reticulocyte count, the number of platelets, and red cell mass, and increases in the numbers of leucocytes, neutrophils, monocytes, large unstained cells (LUC), basophils and/or the levels of fibrinogen. All changes fully reversed by the end of the recovery phase.

Test item-related changes in hematological and coagulation parameters, group 7 compared to the control group in %		
Parameter	**Group 7: 100 µg BNT162b2/animal**	
	Males	**Females**
Test day 4		
Reticulocytes (relative)	-74.3**	-47.7**
Reticulocytes (absolute)	-72.1**	-48.2**
Large unclassified cells (LUC), abs.	+295.5**	+319.5**
Basophils (Baso), abs.	+150.0**	None
Test day 17		
Hemoglobin (HGB)	-9.1**	-12.7**
Erythrocytes (RBC)	None	-9.8**
Hematocrit (HCT)	-11.9**	-13.5**
Leucocytes (WBC)	+118.7**	+111.0**
Platelets (PLT)	-29.2**	-34.1**
Neutrophils (Neut), abs.	+605.8**	+679.8**
Eosinophils (Eos), abs.	+419.3**	+509.6**
Large unclassified cells, (LUC) abs.	+685.2**	+594.8**
Basophils (Baso), abs.	+146.7**	+105.3*
Fibrinogen	+205.2**	+160.2**

abs. = absolute. None = No test item-related change. */** = Statistically significant at $p \leq 0.01$ / $p \leq 0.05$ (based on numerical data, not on percent difference).

Table 13: test article-related changes in hematological and coagulation parameters for the treatment with BNT162b2

Acute phase protein levels:

		(b) (4) Parameters-Male		(b) (4) Parameters-Female	
		Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]	Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]
Group 1: Control	Mean	64658.6	39774.6	79798.8	18098.2
	SD	6727.8	3460.7	17269.9	5486.8
	N	5	5	5	5
		-	-		
Group 2: (b) (4)	Mean	(b) (4)			
	SD				

		(b) (4) Parameters-Male		(b) (4) Parameters-Female	
		Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]	Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]
animal T. item 1	N %Diff	(b) (4)			
Group 3: (b) (4) / animal T. item 1	Mean SD N %Diff				
Group 4: (b) (4) / animal T. item 3	Mean SD N %Diff				
Group 5: (b) (4) / animal T. item 3	Mean SD N %Diff				
Group 6: (b) (4) / animal T. item 5	Mean SD N %Diff				
Group 7: 100 µg/ animal T. item 4	Mean SD N %Diff	446781.0** 64502.0 5 591.0	2159010.0** 78652.0 5 5328.1	445614.0** 27975.1 5 458.4	1362630.0** 257962.6 5 7429.1

[a] - Anova & Dunnett (Log): ** = $p \leq 0.01$
[a1] - Anova & Dunnett (Rank): ** = $p \leq 0.01$

Table 14: Acute phase protein levels, day 4 relatives to start date

At study day 4, alpha1-acid glycoprotein and alpha2 macroglobulin levels were increased significantly ($p \leq 0.01$) in all treated male's and female's groups.

		(b) (4) Parameters-Male		(b) (4) Parameters-Female	
		Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a]	Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a]
Group 6: (b) (4) / animal T. item 5	Mean SD N	(b) (4)			

[a] - Anova & Dunnett (Log): ** = $p \leq 0.01$

Table 15: Acute phase protein levels, day 10 relatives to start date

		(b) (4) Parameters-Male		(b) (4) Parameters-Female	
		Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]	Alpha1-acid Glycoprotein (ng/mL) [a]	Alpha2 Macroglob. (ng/mL) [a1]
Group 1: Control	Mean	50334.7	-	52001.7	-
	SD	11962.9	-	10058.1	-
	N	10	-	10	-
	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)			
	SD				
	N				
	%Diff				
Group 3: (b) (4) / animal T. item 1	Mean				
	SD				
	N				
	%Diff				
Group 4: (b) (4) / animal T. item 3	Mean				
	SD				
	N				
	%Diff				
Group 5: (b) (4) / animal T. item 3	Mean				
	SD				
	N				
	%Diff				
Group 7: 100 µg/ animal T. item 4	Mean	1043631.5**	-	826053.0**	-
	SD	80157.0	-	274115.3	-
	N	10	-	10	-
	%Diff	1973.4	-	1488.5	-

[a] - Anova & Dunnett (Log): ** = $p \leq 0.01$
[a1] - Anova & Dunnett (Rank): ** = $p \leq 0.01$

Table 16: Acute phase protein levels, day 17 relatives to start date

At study days 10 and 17, alpha1-acid glycoprotein levels were increased significantly ($p \leq 0.01$) in all treated male's and female's groups.

Cytokine levels:

Sex: Male		Day 1 Relative to Start Date (PreDs) Cytokine Levels				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a]	IL-6 (pg/mL) [a]	IL-10 (pg/mL) [a]
Group 1: Control	Mean	7.23	7.10	12.60	3.00	9.90
	SD	5.60	0.00	0.00	0.00	0.00
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					
Day: 1 Relative to Start Date (6 h pa)						
Group 1: Control	Mean	99.17	66.10	349.93	12.33	212.37
	SD	7.60	14.69	115.46	8.31	116.87
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett(Log)
[a2] - Anova & Dunnett(Rank): n - Inappropriate for statistics

Table 17: Cytokine levels in males at study day 1

(b) (4)

Sex: Male		Day 8 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a]	IL-6 (pg/mL) [a]	IL-10 (pg/mL) [a]
Group 1: Control	Mean	109.77	92.47	447.53	14.57	365.60
	SD	20.35	19.99	87.14	16.21	74.22
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					
Sex: Male		Day 8 Relative to Start Date (6 h pa)				
Group 1: Control	Mean	88.43	56.80	269.07	4.50	220.07
	SD	19.95	20.82	111.47	2.60	106.23
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 18: Cytokine levels in males at study day 8

(b) (4)

Sex: Male		Day 15 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a]
Group 1: Control	Mean	84.90	66.80	269.17	3.00	178.57
	SD	61.87	52.44	231.66	0.00	147.46
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					
Sex: Male		Day 15 Relative to Start Date (6 h pa)				
Group 1: Control	Mean	125.33	82.30	381.77	3.53	238.63
	SD	24.16	36.60	149.65	0.92	102.97
	N	3	3	3	3	3
	-	-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 19: Cytokine levels in males at study day 15

(b) (4)

Sex: Male		Cytokine Levels				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a1]
Group 1: Control	Mean	4.00	7.10	12.60	3.00	9.90
	SD	0.00	0.00	0.00	0.00	0.00
	N	3	3	3	3	3
		-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 20: Cytokine levels in males at study day 17 relatives to start date (48h pa)

(b) (4)

Sex: Female		Day 1 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a1]	IL-1beta (pg/mL) [a1]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a1]
Group 1: Control	Mean	30.67	28.57	119.00	3.00	71.90
	SD	46.19	23.95	135.10	0.00	107.39
	N	3	3	3	3	3
		-	-	-	-	-
Group 2: (b) (4) µg/ animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) µg/ animal T. item 3	Mean					
	SD					
	N					
	%Diff					
Day: 1 Relative to Start Date (6 h pa)						
Group 1: Control	Mean	86.50	65.83	345.70	5.77	168.03
	SD	8.29	29.96	188.07	3.19	78.07

Sex: Female		Day 1 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a1]	IL-1beta (pg/mL) [a1]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a1]
	N	3 -	3 -	3 -	3 -	3 -
Group 2: (b) (4) µg/ animal T. item 1	Mean SD N %Diff	(b) (4)				
Group 4: (b) (4) µg/ animal T. item 3	Mean SD N %Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 21: Cytokine levels in females at study day 1

Sex: Female		Day 8 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a1]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a1]
Group 1: Control	Mean SD N	23.27 31.91 3 -	12.80 9.87 3 -	48.37 61.95 3 -	3.00 0.00 3 -	17.80 13.68 3 -
Group 2: (b) (4) µg/ animal T. item 1	Mean SD N %Diff	(b) (4)				
Group 4: (b) (4) µg/ animal T. item 3	Mean SD N %Diff					
Day 8 Relative to Start Date (6 h pa)						
Group 1: Control	Mean SD N	77.80 18.19 3 -	43.67 19.70 3 -	213.37 99.74 3 -	3.00 0.00 3 -	125.70 98.90 3 -
Group 2: (b) (4) µg/ animal T. item 1	Mean SD N %Diff	(b) (4)				
Group 4: (b) (4) µg/ animal	Mean SD N					

T. item 3	%Diff	(b) (4)

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 22: Cytokine levels in females at study day 8

(b) (4)

Sex: Female		Day 15 Relative to Start Date (PreDs)				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a]
Group 1: Control	Mean	37.33	26.27	116.57	3.00	66.90
	SD	57.74	33.20	180.08	0.00	98.73
	N	3	3	3	3	3
		-	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) / animal T. item 3	Mean					
	SD					
	N					
	%Diff					
Sex: Female		Day 15 Relative to Start Date (6 h pa)				
Group 1: Control	Mean	121.37	90.97	420.53	3.27	230.10
	SD	18.61	29.50	143.71	0.46	89.38
	N	3	3	3	3	3
		-	-	-	-	-
Group 2: (b) (4) µg/ animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) µg/ animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 23: Cytokine levels in females at study day 15

(b) (4)

Sex: Female		Cytokine Levels				
		IFN-gamma (pg/mL) [a]	TNF-alpha (pg/mL) [a]	IL-1beta (pg/mL) [a1]	IL-6 (pg/mL) [a1]	IL-10 (pg/mL) [a]
Group 1: Control	Mean	32.37	20.03	77.83	3.00	45.87
	SD	49.13	22.40	112.99	0.00	62.30
	N	3	3	3	3	3
		-	-	-	-	-
Group 2: (b) (4) µg/ animal T. item 1	Mean	(b) (4)				
	SD					
	N					
	%Diff					
Group 4: (b) (4) µg/ animal T. item 3	Mean					
	SD					
	N					
	%Diff					

[a] - Anova & Dunnett
[a1] - Anova & Dunnett (Log)
[a2] - Anova & Dunnett (Rank): n - Inappropriate for statistics

Table 24: Cytokine levels in females at study day 17 relatives to start date (48h pa)

(b) (4)

Urinalysis:
No test article-related effects on the urinalysis tests were reported.

Sex: Male		Urinalysis		
		Specific Gravity (g/mL) [a]	pH [a]	Urine Volume - relative - (mL/kg b.w./24 h) [a]
Group 6: (b) (4)/ animal T. item 5	Mean	(b) (4)		
	SD			
	N			

Table 25: Urinalysis results in males at day 10 relatives to start date

Sex: Male		Urinalysis		
		Specific Gravity (g/mL) [a]	pH [a1]	Urine Volume - relative - (mL/kg b.w./24 h) [a1]
Group 1: Control	Mean	1.0309	6.55	45.80
	SD	0.0057	0.20	5.62
	N	10	10	10
	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)		
	SD			
	N			
	%Diff			
Group 3: (b) (4) / animal T. item 1	Mean			
	SD			
	N			
	%Diff			
Group 4: (b) (4) / animal T. item 3	Mean			
	SD			
	N			
	%Diff			
Group 5: (b) (4) / animal T. item 3	Mean			
	SD			
	N			
	%Diff			
Group 7: 100 µg/ animal T. item 4	Mean	1.0463**	6.35	31.67**
	SD	0.0122	0.27	9.65
	N	10	10	10
	%Diff	1.5	-3.1	-30.9

Table 26: Urinalysis results in males at day 17 relatives to start date

Specific gravity was increased significantly in groups (b) (4) 7 males at study day 17. Urine volume was decreased significantly in groups (b) (4) 7 males at study day 17.

Sex: Female		Urinalysis		
		Specific Gravity (g/mL) [a]	pH [a]	Urine Volume - relative - (mL/kg b.w./24 h) [a]
Group 6: (b) (4) / animal T. item 5	Mean	(b) (4)		
	SD			
	N			

Table 27: Urinalysis results in females at day 10 relatives to start date

Sex: Female		Urinalysis		
		Specific Gravity (g/mL) [a]	pH [a1]	Urine Volume - relative - (mL/kg b.w./24 h) [a1]
Group 1: Control	Mean	1.0349	6.26	45.54
	SD	0.0047	0.26	10.71
	N	10	10	10
	-	-	-	-
Group 2: (b) (4) / animal T. item 1	Mean	(b) (4)		
	SD			
	N			
	%Diff			
Group 3: (b) (4) / animal T. item 1	Mean			
	SD			
	N			
	%Diff			
Group 4: (b) (4) / animal T. item 3	Mean			
	SD			
	N			
	%Diff			
Group 5: (b) (4) / animal T. item 3	Mean			
	SD			
	N			
	%Diff			
Group 7: 100 µg/ animal T. item 4	Mean	1.0400	6.26	38.35
	SD	0.0099	0.20	15.62
	N	10	10	10
	%Diff	0.5	0.0	-15.8

Table 28: Urinalysis results in females at day 17 relatives to start date

Specific gravity was (b) (4) significantly in group (b) (4) females at study day 17. Urine volume was decreased, not to significance, in groups (b) (4) 7 females at study day 17.

Systemic toxicity:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, food consumption, body temperature, ophthalmic changes, urinalysis, or auditory examination were reported.

Treatment period for BNT162a1 - Groups 2 and 3

(b) (4)

(b) (4)

Treatment period for BNT162b1 - Groups 4 and 5

(b) (4)

Treatment period for BNT162c1 - Group 6

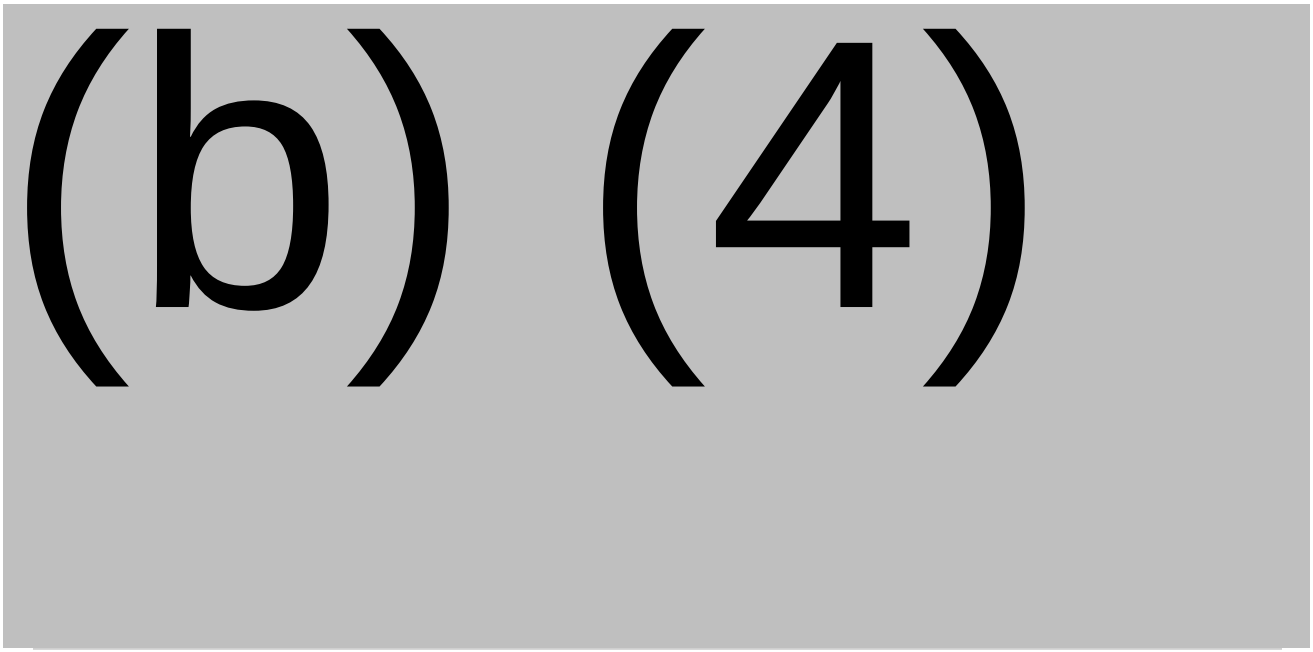
(b) (4)

Treatment period for BNT162b2 - Group 7

On study days 1, 8, and/or 15, very slight to severe (rarely) edema were reported for all animals following the 1st, 2nd, and/or 3rd injection of 100 μg BNT162b2/animal. All edema reported after the 1st or 2nd injection had subsided by 96 hr's post administration. In addition, a few female animals revealed very slight erythema following 24 to 96 hr's following the 1st or 2nd injection.

Skin reddening (scored as "severe" erythema) was reported in individual male and female animals at 144 hr's after the 2nd injection only but was resolved prior to the 3rd injection.

The macroscopic inspection at necropsy revealed an indurated and/or thickened injection site for 7 of 10 male and 9 of 10 female main study animals treated with 100 µg BNT162b2/animal.



Local reactions were slight after first immunization but more pronounced after boost with a reduced immunization interval.

Histopathological examination of injection sites at treatment period
Characterized mostly by moderate inflammation (up to marked) in males and moderate inflammation in females, the histopathological examination revealed test article-related injection site findings in all groups. The most severe findings were reported consistently in animals administered (b) (4) /animal and 100 µg BNT162b2/animal, followed by animals administered (b) (4) /animal. The inflammation was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis. Injection site inflammation was associated with mostly moderate edema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. Skin ulceration (mild and moderate) was reported in some males and females administered either (b) (4) /animal and one animal administered (b) (4) /animal. Inflammation extended into tissues adjacent to the injection site, including mammary tissue, perineural tissue of sciatic nerve, tissue around the femur / knee and to the draining lymph node (iliac). No notable injection site findings in the control group was reported.

Body weight gain:
Test article-related treatment decreases in male's body weight gains were reported in all groups. In females, this effect was less severe in groups (b) (4) . The decrease in groups (b) (4) 7 body weight gains were higher. The results of the body weight gains are reported in the figures below.

Figure 10: Body weight gain of male rats

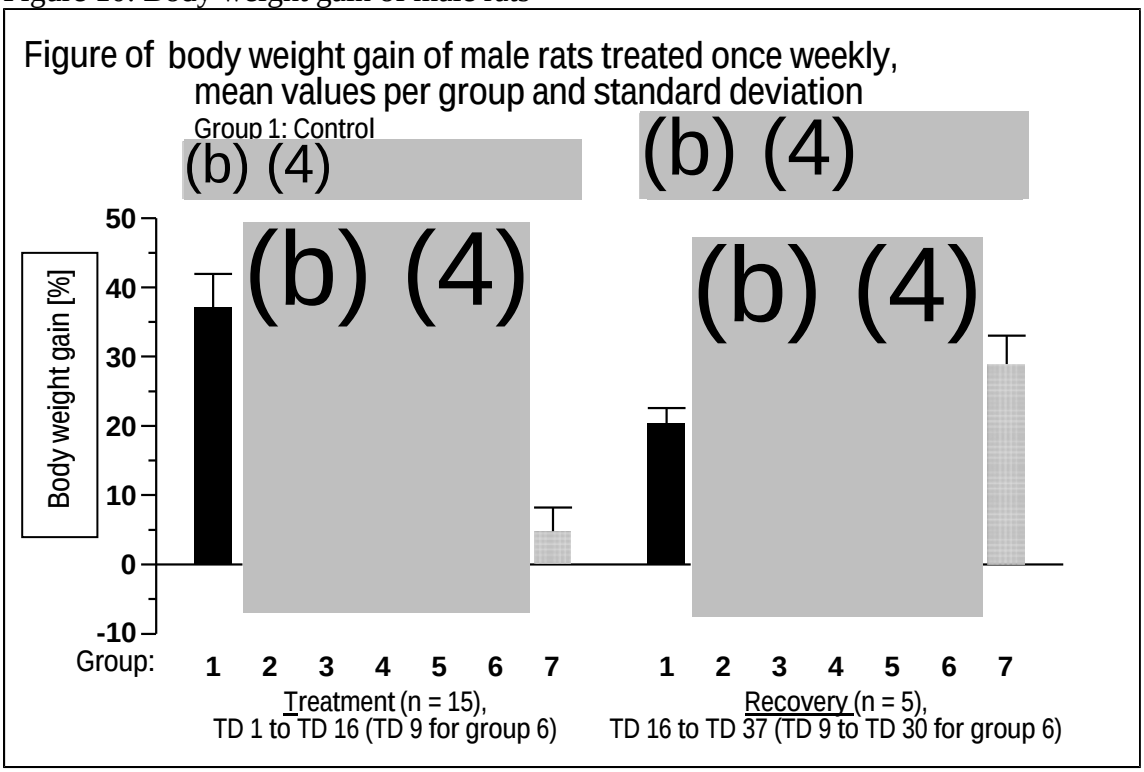
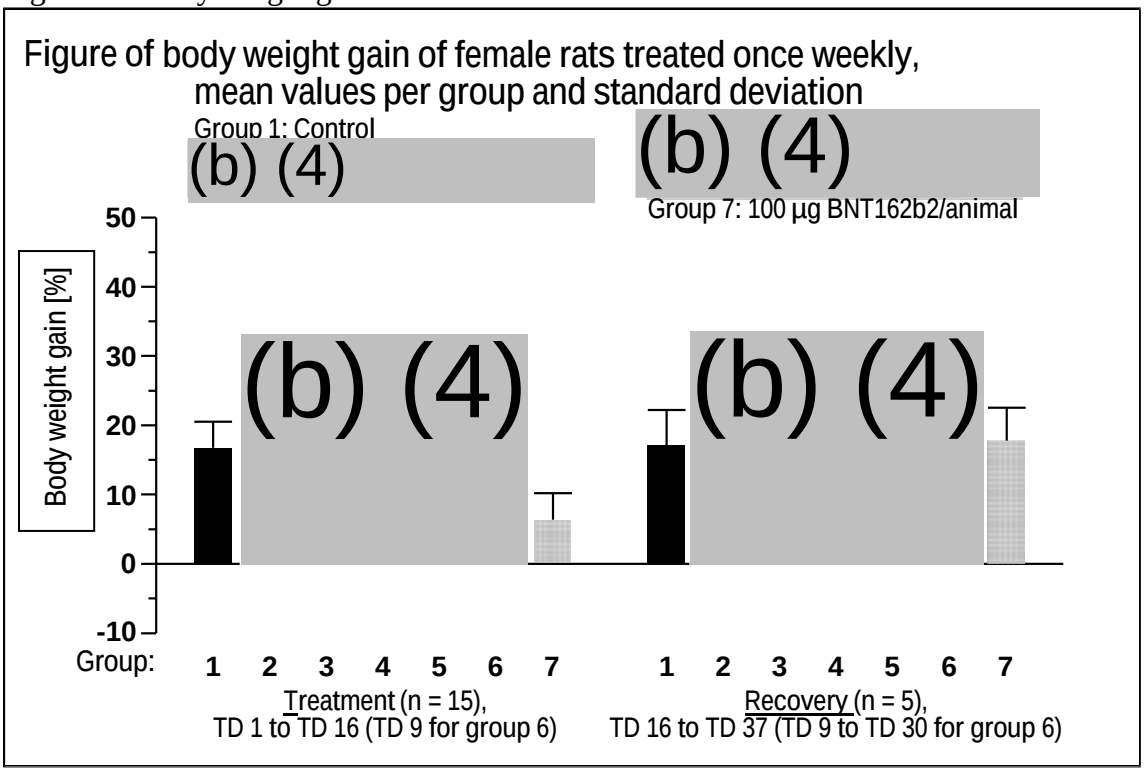


Figure 11: Body weight gain of female rats



Organ Weight:

SEX	Males SD (10/17)						
GROUPS	1 (CONTROL)	2	3	4	5	6	7
NUMBER OF ANIMALS	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]
BODY WEIGHT (terminal)	NC/327	(b) (4)					NC/299
BRAIN	NC/2.00						NC/1.94
ADRENALS-LEFT	NC/0.038						NC/0.043
ADRENALS-RIGHT	NC/0.035						NC/0.037
EPIDIDYMIDES-L	NC/0.457						NC/0.55*
EPIDIDYMIDES-R	NC/0.419						NC/0.51*
HEART	NC/1.14						NC/1.14
KIDNEYS-L	NC/1.426						NC/1.390
KIDNEYS-R	NC/1.479						NC/1.431
LIVER	NC/13.02						NC/12.16
LUNGS	NC/1.936						NC/1.877
CERV LYMPH NODES	NC/0.021						NC/0.016
INGUINAL LYMPH NODES	NC/NC						NC/NC
MANDIBULAR LYMPH NODES	NC/NC						NC/NC
MESENTERIC LYMPH NODES	NC/0.033						NC/0.050
POPLITEAL LYMPH NODES	NC/NC						NC/NC
PROSTATE	NC/0.927						NC/0.813
SPLEEN	NC/0.838						NC/1.049**
TESTES-L	NC/1.80						NC/1.84
TESTES-R	NC/1.78						NC/1.82
PITUITARY	NC/0.013						NC/0.011
THYROID and PARATHYROID	NC/0.013						NC/0.011
THYMUS	NC/0.538						NC/0.388**
OVARIES							
UTERUS							

NC = Not collected. L = Left; R = Right. CERV = Cervical. [a] - Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$

Table 29: Male's organ weights results. Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

Study day 17 male's results:
Body weight was (b) (4) in group (b) (4). Left adrenal weight was (b) (4) in groups (b) (4). Right adrenal weight was increased 13% in groups (b) (4) 7. Right adrenal weight was (b) (4) in groups (b) (4). Right adrenal weight was (b) (4) in groups (b) (4), respectively. Left epididymides weight was increased (b) (4), and 20% in groups (b) (4) and 7, respectively. Right epididymides weight was increased (b) (4) and 22% in groups (b) (4) and 7, respectively. Liver weight was (b) (4) in group (b) (4). Cervical lymph node's weight was decreased 24% in group 7. Mesenteric lymph node's weight was increased (b) (4) and 52% in groups (b) (4) and 7, respectively. Prostate weight was decreased (b) (4) and 12% in group (b) (4) and 7, respectively. Spleen weight was increased (b) (4) and 25% in groups (b) (4) and 7, respectively. Thymus weight was decreased (b) (4) and 28% in groups (b) (4) and 7, respectively.

SEX	Females SD (10/17)						
GROUPS	1 (CONTROL)	2	3	4	5	6	7
NUMBER OF ANIMALS	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]
BODY WEIGHT (terminal)	NC/221	(b) (4)					NC/219
BRAIN	NC/1.86						NC/1.87
ADRENALS-L	NC/0.045						NC/0.049
ADRENALS-R	NC/0.044						NC/0.049
EPIDIDYMIDES-L							
EPIDIDYMIDES-R							
HEART	NC/0.914						NC/0.866
KIDNEYS-L	NC/0.938						NC/1.009
KIDNEYS-R	NC/0.989						NC/1.057
LIVER	NC/8.35						NC/9.95**
LUNGS	NC/1.333						NC/1.524
CERV LYMPH NODES	NC/0.016						NC/0.017
INGUINAL LYMPH NODES	NC/NC						NC/NC
MANDIBULAR LYMPH NODES	NC/NC						NC/NC
MESENTERIC LYMPH NODES	NC/0.034						NC/0.037
POPLITEAL LYMPH NODES	NC/NC						NC/NC
PROSTATE							
SPLEEN	NC/0.595						NC/0.957**
TESTES-L							
TESTES-R							
PITUITARY	NC/0.015						NC/0.014
THYROID and PARATHYROID	NC/0.013						NC/0.011
THYMUS	NC/0.456						NC/0.390

SEX	Females SD (10/17)						
GROUPS	1 (CONTROL)	2	3	4	5	6	7
NUMBER OF ANIMALS	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]	10/10 [a]
OVARY-L	NC/0.054	(b) (4)					NC/0.049
OVARY-R	NC/0.058						NC/0.056
UTERUS	NC/NC						NC/NC

NC = Not collected. L = Left; R = Right. CERV = Cervical. [a] - Anova & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$

Table 30: Female's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed both as organ weight from animals taken at the end of the terminal phase and recovery phase of the study (main phase organ weight/recovery phase organ weight).

Study day 17 female's results:

Left and right adrenal weight was (b) (4) in group (b) (4). Left kidney weight was (b) (4) in group (b) (4). Liver weight was increased (b) (4) 19% in groups (b) (4) 7, respectively. Lungs weight was increased (b) (4) 14% in groups (b) (4) 7, respectively. Cervical lymph node's weight was increased (b) (4) in groups (b) (4), respectively. Mesenteric lymph node's weight was (b) (4) in group (b) (4). Mesenteric lymph node's weight was (b) (4) in groups (b) (4), respectively. Spleen weight was increased (b) (4) 61% in groups (b) (4) 7, respectively. Thyroid weight was decreased (b) (4) 15% in groups (b) (4) 7, respectively. Thymus weight was decreased (b) (4) 14% in groups (b) (4) 7, respectively.

Gross pathology:

Test article-related findings in all groups included injection site findings, enlarged iliac lymph nodes, and enlarged spleen. All other findings were considered incidental.

Groups	Findings
1M	Emphysematous-lungs (1/10); reddened thymus (1/10)
2M	(b) (4)
3M	
4M	
5M	

Groups	Findings
6M	(b) (4)
7M	Indurated injections site I+II (5/10); enlarged iliac lymph nodes (5/10); enlarged renal lymph nodes (1/10); enlarged spleen (2/10); thickened injection sites I+II (1/10)

Table 31: Male's gross pathology results.

Groups	Findings
1F	No findings
2F	(b) (4)
3F	
4F	
5F	
6F	
7F	Indurated injections site I (3/10); indurated injections site I+II (4/10); enlarged iliac lymph nodes (7/10); enlarged spleen (7/10); thickened injection sites I (2/10); muscle jellied [adhered to sciatic nerve and bone] at injection site I (1/10); dilated uterus [filled with clear liquid] (1/10); sciatic nerve adhered to injection site I (2/10)

Table 32: Female's gross pathology results.

Microscopic findings:

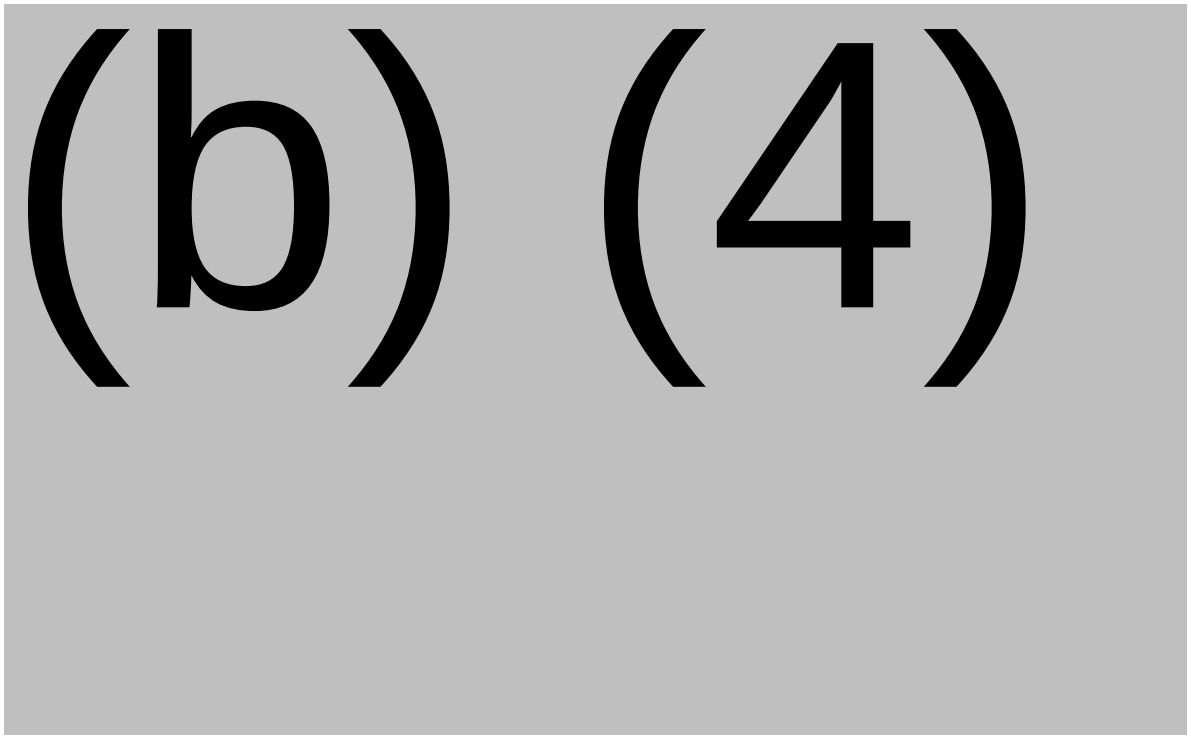
Terminal sacrifice

Inflammation at the injection site and surrounding tissues, increased cellularity of germinal centers and increased plasma cells in the draining (iliac) lymph node, increased cellularity (hematopoiesis) in the bone marrow and spleen, and vacuolation of hepatocytes in the portal regions were the test article-related microscopic findings reported at the end of dosing period. At the end of the 3-week recovery phase, all microscopic findings were partially or fully recovered.

In all groups, test article-related injection site reactions were reported. Site reactions were mostly characterized by moderate inflammation (up to marked) in males and moderate inflammation in females. In groups (b) (4) 7 ((b) (4) 100 µg BNT162b2/animal), the most severe findings were consistently reported. Followed by the animals administered (b) (4) /animals. The inflammation at the injection site was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis. Injection site inflammation was associated with mostly moderate edema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. In some males and females treated with either (b) (4) /animal and one animal administered (b) (4) animal, skin ulceration (mild and moderate) was reported. At the end of the 3-week recovery phase, injection site findings were partially recovered. The inflammation at the injection sites were extended into tissues adjacent to it. The adjacent tissues included mammary tissue, perineural tissue of sciatic nerve, tissue around the femur/knee and to the draining lymph node (iliac). At the end of the 3-week recovery phase, these findings were mostly recovered.

In the draining (iliac) lymph node, test article-related findings were characterized by increased cellularity of the follicular germinal centers and increased plasma cells (plasmacytosis) and were variably present in all groups. In all test article-treated groups, minimal to mild increases in the cellularity of bone marrow were reported. They were likely secondary to inflammation-related platelet activation and consumption. Also, extramedullary hematopoiesis in the spleen were reported. A test article-related vacuolation of hepatocytes in the portal regions of the liver was reported in all groups.

A few other minor microscopic changes were recorded for other organs and were not considered test article-related. All changes are regarded to be spontaneous in nature being within the normal background pathology commonly reported in rats of this strain and age.



(b) (4)

…/... number of animals affected per number of animals examined
* significantly different from control ($p \leq 0.05$)
** significantly different from control ($p \leq 0.01$)

Table 33: Incidences of test article-related microscopic findings for the animals treated with BNT162a1

(b) (4)

(b) (4)

…/... number of animals affected per number of animals examined
* significantly different from control ($p \leq 0.05$)
** significantly different from control ($p \leq 0.01$)

Table 34: Incidences of test article-related microscopic findings for the animals treated with BNT162b1

(b) (4)

Incidences of test item-related microscopic findings in male and female main study animals after terminal sacrifice on test day 10 (group 6) or test day 17 (group 7)			
Organ / Finding	**BNT162 c1**	**BNT162 b2**	
	(b) (4)	**Group 7: 100 µg/animal**	
		Males	**Females**
Perineural tissue of sciatic nerve:	(b) (4)	10/10**	10/10**
- Inflammation (perineural)			
Bone, os femoris with joint (surrounding tissue):			
- Inflammation		2/10	9/10**
Mammary gland (Interstitial tissue):			
- Inflammation		2/10	0/10
Lymph node (iliac):			
- Plasmacytosis		10/10**	10/10**
- Inflammation		9/10**	6/10*
- Increased cellularity, germinal center		10/10	10/10**
Skeletal muscle:			
- Infiltration, lymphohistiogranulocyt.		5/10*	0/10
Spleen:			
- Increased haematopoiesis		2/10	8/10**
Liver			
- Vacuolation, hepatocellular, periportal		9/10**	10/10**

…/... number of animals affected per number of animals examined
* significantly different from control ($p \leq 0.05$)
** significantly different from control ($p \leq 0.01$)

Table 35: Incidences of test article-related microscopic findings for the animals treated with BNT162c1 and BNT162b2

Table 36: Microscopic findings at terminal sacrifice

Observations:Neo-PlasticandNonNeo-Plastic----------MALES----------FEMALES----------Removal Reasons: All of those SELECTED Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group 7:
Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group7:

	Control	(b) (4)	100 µg/	Control	(b) (4)	100 µg/
Number of Animals on Study :	15		15	15		15
Number of Animals Completed:	(15)		(15)	(15)		(15)
ADRENAL GLAND, LEFT;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	86.7%		73.3%	80.0%		80.0%
Dilation;vascular	13.3%		20.0%	20.0%		20.0%
ADRENAL GLAND, RIGHT;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	86.7%		73.3%	86.7%		93.3%
Dilation;vascular	13.3%		20.0%	13.3%		6.7%
BONE, OS FEMORIS WITH JOINT;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	100.0%		86.7%	100.0%		40.0%
Inflammation; mixed; surrounding tissue .	0.0%		13.3%	0.0%		60.0%
BONE MARROW, OS FEMORIS WITH JOINT;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	100.0%		33.3%	100.0%		33.3%
IncreasedCellularity	0.0%		66.7%	0.0%		66.7%
CERVIX;						
Examined	(-)		(-)	(15)		(14)
WithinNormalLimits	-		-	80.0%		78.6%
Keratinization;epithelial	-		-	20.0%		21.4%
EPIDIDYMIS, LEFT;						
Examined	(15)		(15)	(-)		(-)
WithinNormalLimits	26.7%		26.7%	-		-
Infiltration,Lymphocytic	73.3%		73.3%	-		-
EPIDIDYMIS, RIGHT;						
Examined	(15)		(15)	(-)		(-)
WithinNormalLimits	33.3%		13.3%	-		-
Infiltration,Lymphocytic	66.7%		86.7%	-		-
HEART;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	100.0%		86.7%	100.0%		100.0%
Infiltration;lymphohistiocytic	0.0%		0.0%	0.0%		0.0%
Infiltration;mixed	0.0%		0.0%	0.0%		0.0%
Infiltration,Lymphocytic	0.0%		13.3%	0.0%		0.0%

Observations:Neo-PlasticandNonNeo-Plastic----------MALES----------FEMALES----------Removal Reasons: All of those SELECTED Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group 7:
Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group7:

	Control	(b) (4)	100 µg/	Control	(b) (4)	100 µg/
Number of Animals on Study :	15		15	15		15
Number of Animals Completed:	(15)		(15)	(15)		(15)
INJECTION SITE I;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	60.0%		0.0%	66.7%		6.7%
Fibrosis; intramuscular / interstitial	0.0%		93.3%	0.0%		93.3%
Fibrosis; inter- / perimuscular	0.0%		100.0%	0.0%		93.3%
Inflammation; lymphohistiocytic; intramuscular / interstitial	0.0%		20.0%	0.0%		26.7%
Inflammation; lymphohistiocytic; inter- / perimuscular	0.0%		33.3%	0.0%		26.7%
Inflammation; mixed; subcutis	0.0%		66.7%	0.0%		66.7%
Inflammation; mixed; intramuscular / interstitial	0.0%		66.7%	0.0%		66.7%
Inflammation; mixed; inter- / perimuscular	0.0%	(b) (4)	66.7%	0.0%	(b) (4)	66.7%
Degeneration; myofiber	6.7%		66.7%	0.0%		66.7%
Edema; subcutis	0.0%		66.7%	0.0%		66.7%
Edema; intramuscular / interstitial	0.0%	(b) (4)	66.7%	0.0%	(b) (4)	66.7%
Edema; inter- / perimuscular	0.0%		66.7%	0.0%		66.7%
Hyperplasia; epidermal	0.0%	(b) (4)	60.0%	0.0%	(b) (4)	66.7%
INJECTION SITE II;						
Examined	(15)		(15)	(15)		(15)
WithinNormalLimits	66.7%		0.0%	66.7%		6.7%
Degeneration; myofiber	0.0%		66.7%	6.7%		66.7%
Regeneration; muscle	0.0%		0.0%	6.7%		0.0%
Hyperplasia; epidermal	0.0%		46.7%	0.0%		60.0%
Scab; epidermal	0.0%		0.0%	0.0%		0.0%
Edema; subcutis	0.0%		66.7%	0.0%		66.7%
Edema; inter- / perimuscular	0.0%		66.7%	0.0%		66.7%
Edema; intramuscular / interstitial	0.0%		66.7%	0.0%		66.7%
Necrosis; myofiber	0.0%		0.0%	0.0%		6.7%
Necrosis; dermis; subcutis	0.0%		0.0%	0.0%		0.0%
Necrosis; traumatic; myofiber	0.0%		0.0%	0.0%		0.0%
Fibrosis; subcutis	0.0%		6.7%	0.0%		0.0%
Fibrosis; inter- / perimuscular	0.0%		100.0%	0.0%		93.3%
Fibrosis; intramuscular / interstitial	0.0%	(b) (4)	86.7%	0.0%	(b) (4)	80.0%

Observations:Neo-PlasticandNonNeo-Plastic----------MALES----------FEMALES----------Removal Reasons: All of those SELECTED Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group 7:
Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group7:

	Control	(b) (4)	100 µg/	Control	(b) (4)	100 µg/
Number of Animals on Study :	15		15	15		15
Number of Animals Completed:	(15)		(15)	(15)		(15)
Inflammation;mixed;subcutis.....	0.0%		66.7%	0.0%		66.7%
Inflammation;mixed;inter-/ perimuscular	0.0%		66.7%	0.0%		66.7%
Inflammation;mixed;intramuscular/ interstitial	0.0%		66.7%	0.0%		66.7%
INTESTINE, RECTUM;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	86.7%		86.7%	80.0%		46.7%
Infiltration,Eosinophilic;increased...	0.0%		0.0%	6.7%		40.0%
Hyperplasia;mucosa-associatedlymphoid tissue	13.3%		13.3%	6.7%		13.3%
KIDNEY, LEFT;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	6.7%		6.7%	0.0%		0.0%
Congestion	93.3%		93.3%	100.0%		100.0%
Basophilia;tubule.....	13.3%		13.3%	13.3%		0.0%
Infiltration,Lymphocytic.....	26.7%		20.0%	6.7%		0.0%
KIDNEY, RIGHT;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	6.7%		0.0%	0.0%		0.0%
Congestion	93.3%		100.0%	100.0%		100.0%
Basophilia;tubule.....	0.0%		26.7%	0.0%		0.0%
Infiltration,Lymphocytic.....	6.7%		6.7%	6.7%		0.0%
LIVER;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	0.0%		0.0%	0.0%		0.0%
Congestion	100.0%		100.0%	100.0%		100.0%
Hematopoiesis;extramedullary.....	13.3%		13.3%	20.0%		33.3%
Infiltration;mixed.....	6.7%		0.0%	0.0%		6.7%
Necrosis	6.7%		6.7%	0.0%		0.0%
Infiltration,Neutrophilic.....	6.7%		0.0%	0.0%		0.0%
Infiltration,Lymphocytic.....	60.0%		33.3%	60.0%		13.3%
Vacuolation;hepatocellular.....	6.7%		0.0%	0.0%		0.0%
Vacuolation;hepatocellular;periportal.	6.7%		60.0%	0.0%		66.7%

Observations:Neo-PlasticandNonNeo-Plastic----------MALES----------FEMALES----------Removal Reasons: All of those SELECTED Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group 7:
Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group7:

	Control	(b) (4)	100 µg/	Control	(b) (4)	100 µg/
Number of Animals on Study :	15		15	15		15
Number of Animals Completed:	(15)		(15)	(15)		(15)
LUNGS WITH BRONCHI; (continued)						
Hemorrhage;acute.....	26.7%		33.3%	6.7%		0.0%
Hyperplasia;bronchial-associated lymphoidtissue.....	46.7%		20.0%	13.3%		33.3%
Infiltration,Eosinophilic;perivascular	20.0%		6.7%	6.7%		53.3%
LYMPH NODE, CERVICAL;						
Examined.....	(13)		(15)	(15)		(14)
WithinNormalLimits.....	0.0%		6.7%	0.0%		0.0%
Histocytosis	100.0%		86.7%	93.3%		92.9%
Erythrophagocytosis	0.0%		0.0%	0.0%		0.0%
Pigmentation;brown;macrophage.....	0.0%		0.0%	0.0%		0.0%
Hemorrhage	0.0%		0.0%	0.0%		0.0%
Plasmacytosis	0.0%		6.7%	0.0%		0.0%
Increased Cellularity; germinal center ..	100.0%		93.3%	86.7%		85.7%
LYMPH NODE, ILIAC;						
Examined.....	(15)		(15)	(15)		(14)
WithinNormalLimits.....	0.0%		0.0%	0.0%		0.0%
Histocytosis	100.0%		93.3%	93.3%		92.9%
Plasmacytosis	0.0%		73.3%	0.0%		100.0%
Infiltration,Eosinophilic.....	6.7%		0.0%	0.0%		0.0%
Hemorrhage;acute.....	0.0%		0.0%	6.7%		0.0%
Inflammation	0.0%		60.0%	0.0%		42.9%
Infiltration;macrophage.....	0.0%		33.3%	0.0%		28.6%
Increased Cellularity; germinal center ..	86.7%		100.0%	46.7%		100.0%
NERVE, SCIATIC;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	100.0%		20.0%	100.0%		26.7%
Inflammation;perineural.....	0.0%		80.0%	0.0%		73.3%
Vacuolation	0.0%		0.0%	0.0%		0.0%
PROSTATE GLAND;						
Examined.....	(15)		(15)	(-)		(-)
WithinNormalLimits.....	80.0%		86.7%	-		-
Infiltration;mixed.....	0.0%		0.0%	-		-
Inflammation;purulent.....	6.7%		0.0%	-		-
Infiltration,Lymphocytic.....	13.3%		13.3%	-		-

Observations:Neo-PlasticandNonNeo-Plastic----------MALES----------FEMALES----------Removal Reasons: All of those SELECTED Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group 7:
Group 1: Group 2: Group 3: Group 4: Group 5: Group 6: Group7:

	Control	(b) (4)	100 µg/	Control	(b) (4)	100 µg/
Number of Animals on Study :	15		15	15		15
Number of Animals Completed:	(15)		(15)	(15)		(15)
SPLEEN;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	20.0%		53.3%	20.0%		13.3%
Congestion	80.0%		40.0%	80.0%		66.7%
Hematopoiesis;increased.....	0.0%		13.3%	0.0%		53.3%
STOMACH, GLANDULAR;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	6.7%		0.0%	6.7%		20.0%
Infiltration,Eosinophilic.....	93.3%		93.3%	93.3%		73.3%
Infiltration,Lymphocytic.....	0.0%		0.0%	0.0%		0.0%
Dilation;glandular.....	0.0%		6.7%	0.0%		13.3%
THYMUS;						
Examined.....	(15)		(15)	(15)		(15)
WithinNormalLimits.....	66.7%		53.3%	46.7%		40.0%
Cyst	0.0%		0.0%	0.0%		0.0%
Hemorrhage;acute.....	33.3%		46.7%	53.3%		60.0%
UTERUS;						
Examined.....	(-)		(-)	(15)		(15)
WithinNormalLimits.....	-		-	100.0%		93.3%
Dilation	-		-	0.0%		6.7%
VAGINA;						
Examined.....	(-)		(-)	(15)		(15)
WithinNormalLimits.....	-		-	73.3%		60.0%
Keratinization;epithelial.....	-		-	26.7%		40.0%

Recovery sacrifice
At the end of the recovery period (day 31 for group (b) (4) and day 38 for all other groups), most of the microscopic findings reported at the injection sites, iliac lymph node, surrounding tissue of the injection sites (surrounding tissue of bone, os femoris with joint; perineural tissue of sciatic nerve; interstitial tissue of mammary gland; skeletal muscle) and spleen were partially or completely recovered in all animals.

Some inflammatory lesions were still reported at the injection sites and the surrounding tissues in some animals. These lesions were less severe (minimal to mild).

The infiltration of macrophages in the iliac lymph nodes of recovery animals were regarded as a consequence of phagocytosis relating to the inflammatory reactions at the injection sites. Test article-related minimal to mild increases in the cellularity of bone marrow and extramedullary hematopoiesis in the spleen was fully recovered at the end of recovery phase.

Test article-related vacuolation of hepatocytes in the portal regions of the liver was fully recovered at the end of recovery phase. The incidence and the severity of the remaining findings were markedly reduced when compared to the main study animals.

Discussion synopsis
Inflammation was generally most at the end of dosing in groups (b) (4) 7. This is followed by (b) (4) /animal group. Ulceration at the injection site was present only in rats administered (b) (4) . The inflammation was partially or fully resolved at the end of the recovery phase.

Increased cellularity of the germinal centers of the draining (iliac) lymph node and plasmacytosis were reported. This is consistent with the anticipated immune activation by the test articles. Increases in bone marrow cellularity (increased hematopoiesis) and extramedullary hematopoiesis in the spleen were reported. This is consistent with a response to inflammation and immune responses induced by the test article.

Test article-related vacuolation of portal hepatocytes was reported in all groups. The vacuolation was unassociated with markers of hepatocyte damage (i.e. ALAT, ASAT) and has been reported in animals administered pegylated compounds only. This finding was fully reversed at the end of the recovery phase.

Body temperature:
No test article-related effects on body temperature were reported.

(b) (4)

(b) (4)

Serology:
In this study the immunogenicity of the administered SARS-CoV-2-S protein targeted RNA vaccines BNT162a1, BNT162b1, BNT162b2, and BNT162c1 was investigated. At study day 10, serum samples were collected from animals treated with BNT162c1 (group 6). At study day 17 serum samples were collected from animals treated with BNT162a1, BNT162b1, and BNT162b2

(groups 2, 3, 4, 5, and 7). Antibody immune response analyzed by S1 domain and RBD sub-domain specific (b) (4) as well as VSV/SARS-CoV-2-S-based pseudovirus neutralization assay (pVNT).

All BNT162 vaccine candidates elicited a SARSCoV- 2-S protein specific antibody response directed against the S1 domain and the RBD sub-domain. Antibody responses translated into neutralizing activity as reported in the VSV/SARS-CoV-2-S pseudovirus neutralization test. BNT162 vaccine candidates showing higher antigen-specific antibody titers also displayed more pronounced virus neutralization effect.

Figure 14: Antibody titer resulting in 50% pseudovirus neutralization activity (pVN50). Individual VNT titers resulting in 50% pseudovirus neutralization (pVN50) are shown by dots; group mean values are indicated by horizontal bars (±SEM, standard error of the mean).

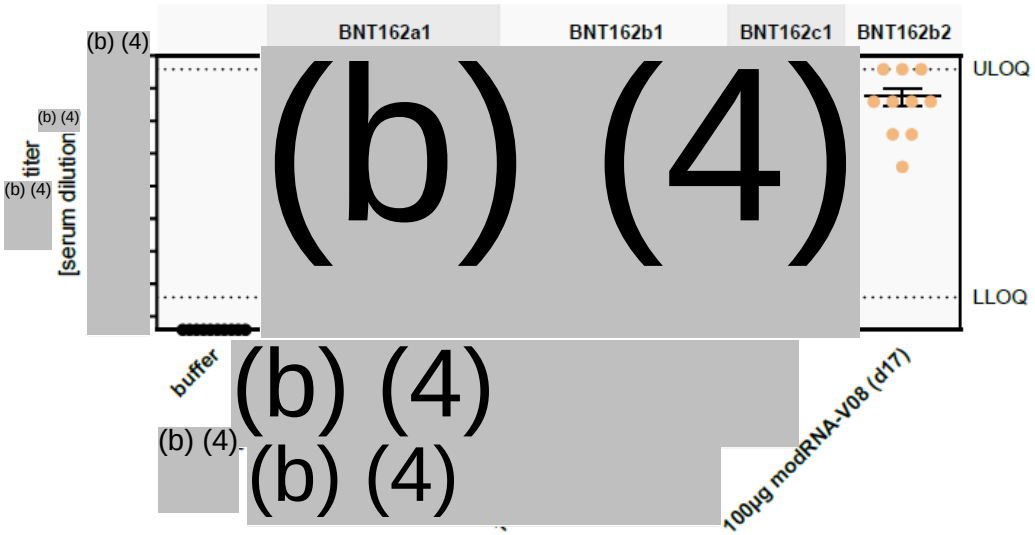
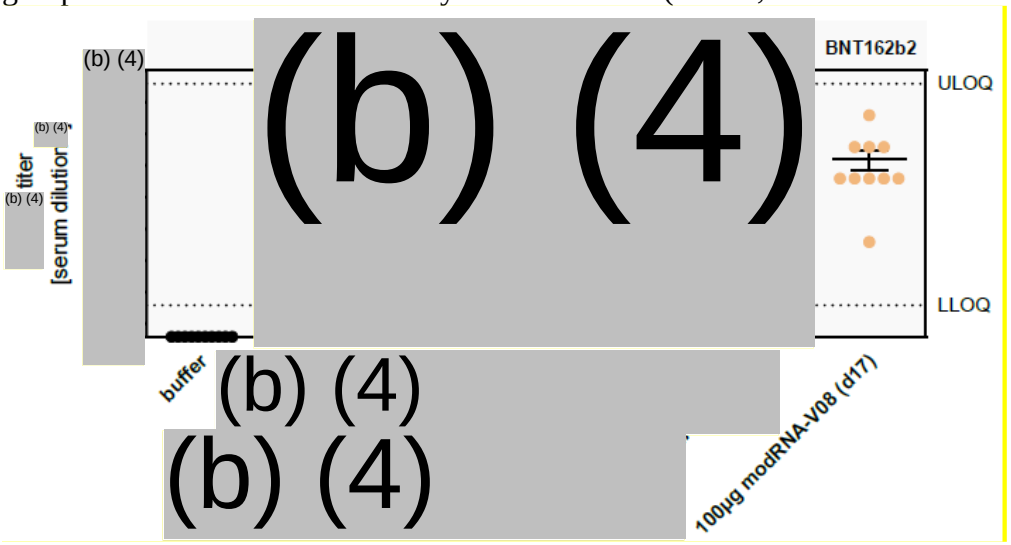


Figure 15: antibody titer resulting in (b) (4) pseudovirus neutralization activity (pVN50). Individual VNT titers resulting in (b) (4) pseudovirus neutralization (pVN(b) (4)) are shown by dots; group mean values are indicated by horizontal bars (±SEM, standard error of the mean).



Test article related effects	Effects considered incidental
↓ Triglycerides ↑ Gamma-GT ↓ Reticulocytes ↓ Platelet ↑ Monocytes ↑ Neutrophils ↑ Eosinophils ↑ Basophils ↑ WBC ↑ LUC ↑ Fibrinogens ↓ PCT% ↑ Alpha1-acid glycoproteins ↑ Alpha2-macroglobulins ↑ Epididymides weight ↑ Mesenteric lymph nodes weight ↑ Spleen weight ↑ Thyroid weight for females Injection site findings (indurated, incrusted, and thickened skin) Enlarged iliac lymph nodes Enlarged spleen ↑ Cellularity of bone marrow Immune responses in groups (b) (4) and 7	↓ Thymus weight IFN-gamma, TNF-alpha, IL-1beta, and IL-10

Table 37: Test article related effects

Assessment:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, food consumption, body temperature, ophthalmic changes, urinalysis, or auditory examination were reported.

A triglyceride is an ester derived from glycerol and three fatty acids.[4] Triglycerides are the main constituents of body fat in humans and animals, as well as vegetable fat.[5] They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils.[6] In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of

[4] *"Nomenclature of Lipids". IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Retrieved 2007-03-08.*
[5] *Nelson, D. L.; Cox, M. M. (2000). Lehninger, Principles of Biochemistry (3rd ed.). New York: Worth Publishing. ISBN 1-57259-153-6.*
[6] *Lampe, M. A.; Burlingame, A. L.; Whitney, J.; Williams, M. L.; Brown, B. E.; Roitman, E.; Elias, M. (1983). "Human stratum corneum lipids: characterization and regional variations". J. Lipid Res. **24**: 120–130. PMID 6833889*

heart disease[7] and stroke.[8] The decrease in triglyceride levels were not considered of any toxicological importance.

Gamma-glutamyl transferase (GGT) is a membrane-bound enzyme catabolizing reduced glutathione to cysteine and glycine in Meister's γ-glutamyl cycle (Orlowski and Meister, 1970).[9] This delivers cysteine for intracellular synthesis of glutathione, the major thiol anti-oxidant. Elevated serum levels of GGT are markers of oxidative stress, resulting from factors including alcohol, heavy metals, cardiovascular disease and diabetes. Furthermore, higher serum levels of GGT, within the normal range, are associated with an increased cancer risk. High levels of GGT seem to increase the risk of progression of high-grade cervical dysplasia to invasive carcinoma.[10]

Reticulocytes are immature red blood cells (RBCs). In the process of erythropoiesis (red blood cell formation), reticulocytes develop and mature in the bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. Like mature red blood cells, in mammals, reticulocytes do not have a cell nucleus.[11] Abnormally low numbers of reticulocytes can be attributed to chemotherapy, aplastic anemia, pernicious anemia, bone marrow malignancies, problems of erythropoietin production, various vitamin or mineral deficiencies (iron, vitamin B_{12}, folic acid), disease states (anemia of chronic disease) and other causes of anemia due to poor RBC production.[12]

The cells that circulate within our blood and bind together when they recognize damaged blood vessels are called **platelets**. The platelets bind to the site of the damaged vessel in any cut, thereby causing a blood clot to stop bleeding. Platelets are literally shaped like small plates in their non-active form. A damaged blood vessel will send out a signal and when platelets receive that signal, they'll respond by traveling to that area and transform into their "active" formation. To make contact with the broken blood vessel, platelets grow long tentacles and then resemble a spider or an octopus. A normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood. Having more than 450,000 platelets is a condition called *thrombocytosis*; having less than 150,000 is known as *thrombocytopenia*. A decrease in platelet levels is called thrombocytopenia. Easy bruising, and frequent bleeding from the gums, nose, or GI tract are the symptoms of *thrombocytopenia*. *Thrombocytopenia happens* when something is preventing your body from producing platelets. There are a wide range of causes, including: medications, an inherited condition, certain types of cancer (such as leukemia or lymphoma), chemotherapy treatment for cancer, kidney infection or dysfunction, or too much alcohol.[13]

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[7] *"Boston scientists say triglycerides play key role in heart health". The Boston Globe. Retrieved 2014-06-18.*

[8] *Drummond, K. E.; Brefere, L. M. (2014). Nutrition for Foodservice and Culinary Professionals (8th ed.). John Wiley & Sons. ISBN 978-0-470-05242-6.*

[9] Orlowski M, Meister A. The γ-glutamyl cycle: a possible transport system for amino acids. PNAS. 1970;67:1248–1255.

[10] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3341856/

[11] https://en.wikipedia.org/wiki/Reticulocyte

[12] https://www.uofmhealth.org/health-library/hw203366

[13] https://www hopkinsmedicine.org/heart_vascular_institute/centers_excellence/women_cardiovascular_health_center/patient_information/health_topics/platelets.html

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in the monocyte count might be related to test article treatment.

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The increase in neutrophils might be related to the immune responses initiated by the test article treatment.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

White blood cells (WBCs) (also called leukocytes or leucocytes) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.[14] The increase in WBC might be related to the immune response induced by the test article treatment.

LUC is a measurement of the large, peroxidase-negative cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. In LUC are found large lymphoid cells, more immature lymphocytes and other cells. If the value is higher than normal, blood counts should be checked under a microscope slide.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

Relative volume of thrombocytes (very large cells in the bone marrow called megakaryocytes)/Plateletcrit (measure of total platelet mass) percent (PCT%) was decreased in groups 3, 5, and 7 males and females at study day 17. This is crucial to normal blood clotting.

Alpha-1-acid glycoprotein (*α_1AGp*,[1] *AGP* or *AAG*), which is modulated by two polymorphic genes, is an acute phase (acute phase protein) plasma alpha-globulin glycoprotein. It has a normal plasma concentration between 0.6-1.2 mg/mL (1-3% plasma protein) and is synthesized primarily in hepatocytes (5). Plasma levels are affected by pregnancy, burns, certain drugs, and certain diseases, particularly HIV (5). The function of alpha-1-acid glycoprotein is to act as a carrier of basic and neutrally charged lipophilic compounds. It is known as the primary carrier of basic (positively charged) drugs (whereas albumin carries acidic (negatively charged) and neutral drugs), steroids, and protease inhibitors (5, 6). AGP shows a complex interaction with thyroid homeostasis. Alpha-1-acid glycoprotein (in low concentrations) was reported to stimulate the

[14] *Maton, D., Hopkins, J., McLaughlin, Ch. W., Johnson, S., Warner, M. Q., LaHart, D., & Wright, J. D., Deep V. Kulkarni (1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall. ISBN 0-13-981176-1.*

thyrotropin (TSH) receptor and intracellular accumulation of cyclic AMP. However, high AGP concentrations inhibited TSH signaling (7, 8). Alpha-1-acid glycoprotein has been identified as one of four potentially useful circulating biomarkers for estimating the five-year risk of all-cause mortality (the other three are albumin, very low-density lipoprotein particle size, and citrate) (9). Alpha-1-acid glycoprotein increases in obstructive jaundices while diminishes in hepatocellular jaundice and in intestinal infections.[15]

Alpha-2-macroglobulin (α2M) is a large plasma protein found in the blood, mainly produced by the liver, and also locally synthesized by macrophages, fibroblasts, and adrenocortical cells. It acts as an antiprotease and is able to inactivate an enormous variety of proteinases. It functions as an inhibitor of fibrinolysis by inhibiting plasmin and kallikrein and as an inhibitor of coagulation by inhibiting thrombin. Because it also binds to numerous growth factors and cytokines, such as platelet-derived growth factor, basic fibroblast growth factor, TGF-β, insulin, and IL-1β, it may act as a carrier protein. In the nephrotic syndrome when other lower molecular weight proteins are lost in the urine, the concentration of alpha-2-macroglobulin rises 10-fold or more[16].

The epididymis is a tube that connects a testicle to a vas deferens in the male reproductive system. It is present in all male reptiles, birds, and mammals. It is a single, narrow, tightly-coiled tube connecting the efferent ducts from the rear of each testicle to its vas deferens. An inflammation of the epididymis is called epididymitis. It is much more common than testicular inflammation, termed orchitis.[17]
The increases in the weights of mesenteric lymph nodes and the enlargement of the iliac lymph nodes might be related to the immune response due to test article treatment.

The external iliac lymph nodes are eight to ten in number, that lie along the external iliac vessels. They are arranged in three groups, one on the lateral, another on the medial, and a third on the anterior aspect of the vessels; the third group is, however, sometimes absent. Their principal afferents are derived from the inguinal lymph nodes, the deep lymphatics of the abdominal wall below the umbilicus and of the adductor region of the thigh, and the lymphatics from the glans penis, glans clitoris, the membranous urethra, the prostate, the fundus of the urinary bladder, the cervix uteri, and upper part of the vagina[18].

Spleen weight increase might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system[19]. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for

[15] https://en.wikipedia.org/wiki/Orosomucoid
[16] https://en.wikipedia.org/wiki/Alpha-2-Macroglobulin
[17] https://en.wikipedia.org/wiki/Epididymis
[18] https://en.wikipedia.org/wiki/External_iliac_lymph_nodes
[19] Spleen, Internet Encyclopedia of Science.

removal[20]. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

The thyroid gland controls how quickly the body makes proteins and uses energy. And, controls how sensitive the body is to other hormones. It produces the thyroid hormones [triiodothyronine (T_3) and thyroxine (sometimes referred to as tetraiodothyronine (T_4)]. These hormones regulate the growth and rate of function of many other systems in the body. T_3 and T_4 are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis. Hormonal output from the thyroid is regulated by thyroid-stimulating hormone (TSH) produced by the anterior pituitary. TSH is regulated by thyrotropin-releasing hormone (TRH) produced by the hypothalamus.

Test article-related injection site findings (indurated, incrusted, and thickened skin) were reported. Inflammation is a relatively common occurrence as part of the acute phase response following administration of some vaccines.

In all test article-treated groups, minimal to mild increases in the cellularity of bone marrow were reported. They were likely secondary to inflammation-related platelet activation and consumption.

Test article-related immune responses in groups 2, 3, 4, 5, and 7 were reported.

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum.[21] One of the major characteristics of vertebrate immunology is thymic involution, the shrinking of the thymus with age, resulting in changes in the architecture of the thymus and a decrease in tissue mass.[22] T-cells are named for the thymus where T-lymphocytes migrate from the bone marrow to mature. Its regression has been linked to the reduction in immunosurveillance in the elderly.[23]

No clear important changes in the levels of cytokines (IFN-gamma, TNF-alpha, IL-1beta, and IL-10) were reported.

Adverse gross alteration that could be indicative of systemic or local toxicity was not reported.

Based on the overall findings in this study, it can be concluded that in Wistar rats, repeat dose on study days 1, 8, and 15 had no adverse effects in terms of systemic toxicity at the dose level of

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[20] Mebius RE, Kraal G. (2005). Structure and function of the spleen. Nat Rev Immunol. 5(8):606-16.
[21] https://en.wikipedia.org/wiki/Thymus.
[22] *Shanley D.P.; Danielle A.W.; Manley N.R.; Palmer D.B.; et al. (2009). "An evolutionary perspective on the mechanisms of immunosenescence". Trends Immunol.* ***30*** *(7): 374–381.* doi:10.1016/j.it.2009.05.001. PMID 19541538
[23] *Linton P.J.; Dorshkind K. (2004). "Age-related changes in lymphocyte development and function". Nat. Immunol.* ***5*** *(2): 133–139.* doi:10.1038/ni1033. PMID 14749784

10, 30, or 100 µg/animal. However, due to the significant decrease in the reticulocyte levels, hematology results should be closely monitored during any clinical trial.

GLP study deviations or amendments: Deviations or amendments were not included in this study submission and expected to be included in the final study report.

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes (X) or no ().

Internal Communication:
Due to the significant decreases in the platelet's and reticulocyte's levels, close monitoring to the hematology data in any clinical trial is highly recommended.

Conclusions:
Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect

Study number 2:

Title and study number: 17-day intramuscular toxicity study of BNT162B2 (V9) and BNT162B3C In Wistar Han rats with a 3-week recovery. Study number: 20GR142.

Performing laboratory: Pfizer Worldwide Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT 06340 USA.
Study initiation date: June 23, 2020
Final report date: August 13, 2020

Test article batch/lot:

Test Article	Lot Number	Expiration Date
BNT162b2 (V9)	COVVAC/270320	27 Sep 2020
BNT162b3c	(b) (4)	04 Dec 2020
0.9% sterile saline	(b) (4)	31 Mar 2021

Animal species and strain: Rat/Wistar/(b) (4):WI(Han)
Breeder/supplier: (b) (4)
Number of animal per group and sex: 15/sex/group
Age: 9 weeks
Body weight range:
Males: 243.1 grams - 291.6 grams
Females: 172.9 grams - 209.5 grams
Route and site of administration: Intramuscular (IM)
Volume of injection: 60 µL

Frequency of administration and study duration:
Animals were treated on study days 1, 8, and 15 into the left hindlimb quadriceps muscle

Dose: See study design

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. At the time of submitting this study, stability studies with the first clinical trial material batch have just been started. Up to now no results are available. Stability data will be included in any upcoming amendment. The table below shows the protocol of stability study I for CTM drug substance batches:

Table of protocol of stability study I for CTM drug substance batches at different storage conditions

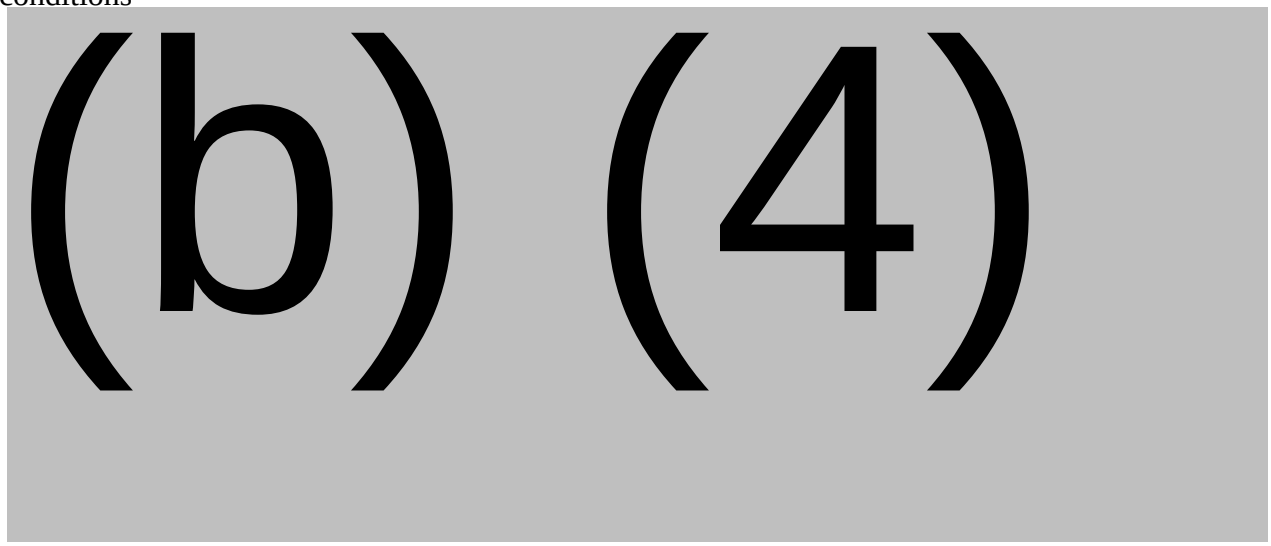

Table 38: Protocol of stability study I for CTM drug substance batches at different storage conditions

Stability of (b) (4) was reported.

Means of administration: Intramuscular (IM)
Report status: Final report

Experimental design:
Animals were randomized and assigned to 3 different groups. Each group consisted of 15/sex/group. 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. Animals were dosed by IM on study days 1, 8, and 15. The details of the study design are listed in the following table:

Table of 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	(b) (4)	(b) (4)	31-45	76-90

a. Each animal received a single intramuscular injection on each dose day.

b. Sterile saline.
c. BNT162b2 (V9).
d. BNT162b3c.

Methods:
Randomization procedure: Yes
Statistical analysis plan: Yes.
The following parameters were evaluated:

General (Cageside) Clinical Observations:	**Days of Study**	**Time Points**
	Prior to the Initiation of Dosing (PID)	Once daily
	Non-dosing Days (Dosing Phase)	Twice daily, except on days when detailed clinical observations were performed, then only once daily
	Dosing Days (Dosing Phase)	Pre dose, except on days that pre dose 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, pre dose on dosing phase days 1, 8, and 15; on dosing phase days 4 and 11 (non-dosing), 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.	

General (Cageside) Clinical Observations:	**Days of Study**	**Time Points**
	Prior to the Initiation of Dosing (PID)	Once daily
	Non-dosing Days (Dosing Phase)	Twice daily, except on days when detailed clinical observations were performed, then only once daily
	Dosing Days (Dosing Phase)	Pre dose, except on days that pre dose 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
Injection Site Scoring (Dermal Assessment):	Injection sites were observed during the dosing phase once pre dose and approximately 4 and 24 hours post dose on all animals. Animals with a score of 2 or greater at 24 hours post dose had additional evaluations at 48- and 72-hours post-dose. Animals with a continued score of 2 or greater at 72 hours post-dose had additional evaluations at 120 and 144 hours post-dose. After dosing on day 15, a 72-hour post dose evaluation was conducted on recovery animals only. Injection site score was recorded according to a standardized rating scale (Draize, 1959)[24]. On dosing phase day 1 (06 Jul 2020), pre dose dermal assessments were collected on all animals for right-side injection sites (non-injection site), and at 4 hours post dose, dermal assessments were collected on animals 1-7, 9 (group 1, males), and 46-58 (group 1, females) for right-side injection sites (non-injection site).	
Body Temperature:	Body temperature was collected on all animals once prior to the initiation of dosing on PID phase day 6, pre-dose on dosing phase days 1, 8, and 15, and at approximately 4- and 24-hours post-dose from all animals.	

Table 39: parameters evaluated

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.

[24] Draize JH. 1959 (2nd printing 1965). Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Dermal Toxicity, pp. 46-59. Published by: The Association of Food and Drug Officials of the United States, Topeka, Kansas.

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.

Table 40: Clinical laboratory measurements

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 -60ºC or lower

PID = Prior to initiation of dosing.
a. Samples collected prior to necropsy.

Table 41: Antibody (Serology) response to vaccine components

Postmortem procedures:
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.

Necropsy, tissue collection, organ weights, macroscopic tissue evaluation, and microscopic examination were performed. Bone marrow smears were collected from all animals.

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

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
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				
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

Table 42: 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				
Bone Marrow, Sternum		X	X	X
Bone, Sternum				

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Brain	X			
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
Stomach				
Testis	X			
Thymus	X			
Tongue				
Trachea				

Tissues Collected	Organs Weighed (All Dose Groups)	Tissues Processed for Slide Preparation (X) Dose Group		
		Group 1	Group 2	Group 3
Ureter				
Urinary Bladder				
Uterus				
Vagina				

Table 43: Tissue collection, organ weights and tissues processed for slide preparation – Recovery phase

Results:
No test article-related mortality was reported.

Clinical chemistry and hematology:
Clinical chemistry

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT STUDY DAY (SD), SEX, DOSE GROUP **(G),** DIRECTION, FOLD CHANGE if great than 1.5 so indicated otherwise ≥ 1.5)**)**	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, potassium, sodium, phosphorus
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR	Alkaline phosphatase (ALP) SD17 F(b) (4) G3	Aspartate aminotransferase (AST or SGOT) Alanine aminotransferase (ALT or SGPT)
B) HEPATOBILIARY		Total bilirubin
ACUTE PHASE REACTANTS	Fibrinogen (also under coagulation)**	
KIDNEY FUNCTION		Creatinine Blood Urea Nitrogen (BUN)
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)	Albumin (A)* GLOB* A/G ratio* A1A GP* A2M*	Total protein Carbon dioxide Globulin Fasting triglycerides Total Cholesterol Creatine kinase (CK) Gamma-GT Lactate dehydrogenase (LDH)

* See table below. ** See table on page 16

Table 44: Serum chemistry results for males and females

Clinical chemistry results showed an (b) (4) in ALP levels in group 3 females at study day 17.

Dosing phase

In groups (b) (4), 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 study days 4 and 17 were reported.

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	(b) (4)	0	30	(b) (4)
ALB (g/dL)						
4D	3.98	0.93x		4.16	0.86x	
17D	3.50	-		3.60	0.85x	
GLOB (g/dL)						
4D	2.13	-		2.10	-	
17D	1.89	1.10x		1.84	1.04x	
AG						
4D	1.88	0.90x		1.98	0.86x	
17D	1.85	0.89x		1.96	0.82x	
A1AGP						
4D	174.358	9.42x		239.774	7.95x	
17D	47.672	38.51x		95.959	15.55x	
A2M						
4D	113.4	20.44x		212.1	3.32x	
17D	14.0	70.76x		33.1	15.74x	

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.

Table 45: Test article-related clinical chemistry parameter effects (mean control values and ratio relative to control mean)

Recovery phase

At study 22 (recovery), all test article related changes were fully reversed, with the exception of higher globulins in group (b) (4), and lower AG ratio in group (b) (4).

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	(b) (4)	0	30	(b) (4)
GLOB (g/dL)						
R22	2.10	1.08x		2.26	1.06x	
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.

Table 46: Test article-related clinical chemistry parameter effects (mean control values and ratio relative to control mean)

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.

Hematology

HEMATOLOGY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL (LIST THE ENDPOINT, STUDY DAY (SD), SEX, DOSE GROUP (G), DIRECTION, FOLD CHANGE if great or less than 1.525, ie, ≥ 1.6 or ≤ 1.6	Not of NOTE
Red blood cells	HCT (%)* Mean Corp. Hb. (MCH)* Mean Corp. Hb. Conc. (MCHC)* Mean Corp. Hb. Conc. (MCHC)* RDW%* Reticulocyte*	Hemoglobin Conc. (Hb) Mean Corp. Volume (MCV) Total Erythrocyte Count (RBC)
White blood cells	Lymphocyte count SD17 F ↑ = 1.7 G2 SD17 (b) (4) WBC* Neutrophil* Monocyte* Eosinophil* Basophil* LUC*	Macrophage Leukocytes
Clotting potential	Fibrinogen*	Activated partial-thromboplastin time clotting time Prothrombin time Platelet count
Others		Bone marrow cytology

* See table on page 16
Table 47: Hematology results for males and females

Terminal phase
Hematology results showed an increase in lymphocyte levels in (b) (4) at study day 17.

Test article-related hematology and coagulation findings were similar in (b) (4) . However, 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) were reported in (b) (4) when compared to group 1. Higher WBC primarily involved higher neutrophils, monocytes and large unstained cells. Higher

[25] With rounding up at the tenth decimal place. Therefore, 1.54 or less becomes 1.5 and is not reported and 1.55 or greater becomes 1.6 and is reported.

eosinophils and basophils were also reported. They were present on days 4 and 17, with higher counts on day 17 than day 4. On study day 17, there were also test article-related higher fibrinogen concentrations in both sexes. Hyper-segmented neutrophils were present on peripheral blood smears of test article-treated animals.

In addition, there were test article-related transiently lower reticulocyte counts on study day 4, and higher reticulocytes on study day 17 (females only). These changes were 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 groups 2 and 3. However, 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) were reported in (b) (4) on days 4 and 17.

Recovery phase

After a 3-weeks recovery phase, all test article-related hematology and coagulation changes were fully reversed, with the exception of higher red cell distribution width.

There were no test article-related findings reported in urinalysis parameters in the dosing or recovery phase.

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	(b) (4)	0	30	(b) (4)
HCT (%)						
4D	48.04	0.90x		44.91	0.93x	
17D	42.61	0.90x		41.67	0.91x	
MCH (pg)						
4D	18.51	-		18.37	-	
17D	18.27	0.96x		18.62	0.97x	
MCHC (g/dL)						
4D	31.24	1.04x		32.34	-	
17D	32.46	-		33.18	-	
RDW (%)						
4D	12.27	-		11.11	-	
17D	11.63	1.21x		11.33	1.18x	
RETIC (10e3/uL)						
4D	392.1	0.27x		301.7	0.43x	
17D	178.8	-		168.9	1.31x	
WBC (10e3/uL)						
4D	7.60	1.41x		6.01	1.30x	
17D	3.84	2.30x		2.16	2.64x	
NEUT (10e3/uL)						
4D	1.083	2.28x		0.920	2.51x	
17D	0.674	6.60x		0.409	6.04x	

Dose (µg RNA/Dose Day)						
Parameter	Males			Females		
Test Article	Vehicle	BNT162b2(V9)	BNT162b3c	Vehicle	BNT162b2(V9)	BNT162b3c
	0	30	(b) (4)	0	30	(b) (4)
MONO (10e3/uL)						
4D	0.109	1.83x		0.093	1.89x	
17D	0.071	3.30x		0.056	2.75x	
EO (10e3/uL)						
4D	0.081	-		0.057	-	
17D	0.056	2.52x		0.029	3.17x	
BASO (10e3/uL)						
4D	0.016	1.88x		0.009	1.89x	
17D	0.003	5.67x		0.001	8.00x	
LUC (10e3/uL)						
4D	0.046	4.07x		0.030	4.20x	
17D	0.026	8.04x		0.010	13.20x	
FIB (mg/dL)						
17D	253.1	2.36x		217.2	2.49x	

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.

Table 48: Test article-related hematology and coagulation parameter effects at main sacrifice (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	(b) (4)	0	30	(b) (4)
RDW (%)						
R22	11.93	1.13x		10.80	1.21x	

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.

Table 49: Test article-related hematology and coagulation parameter effects at recovery phase (mean control values and ratio relative to control mean)

Bone Marrow Assessment

Bone marrow smears were prepared for all animals and were not examined.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, food consumption, body temperature, ophthalmic changes, or urinalysis were reported.

Organ Weight:

In (b) (4), test article-related organ weight differences included higher absolute and relative (to body and brain weight) spleen weights were reported.

No test article-related organ weight changes were reported at the end of the recovery phase.
Table of organ weights results for males

Group Number:		REF				2				3
Dose:		0 µg/day				30 µg/day				(b) (4)
BWT	ABS	**N**	**Mean**	**Ratio**	**SD**	**N**	**Mean**	**Ratio**	**SD**	†
		10	296 06	R REF	16 40	10	271 17	0 92	17 12	
Brain	ABS	10	1 9061	R REF	0 0899	10	1 9159	1 01	0 1445	
	OW:BW	10	0 6449	R REF	0 0335	10	0 7087	1 10	0 0664	†
	OW:BRN	10	1 0000	R REF	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	
	OW:BW	10	0 3936	R REF	0 0536	10	0 3922	1 00	0 0428	
	OW:BRN	10	0 6112	R REF	0 0867	10	0 5570	0 91	0 0756	
Gland, Adrenal	ABS	10	0 0697	R REF	0 0068	10	0 0727	1 04	0 0149	
	OW:BW	10	0 0236	R REF	0 0021	10	0 0267	1 13	0 0045	
	OW:BRN	10	0 0366	R REF	0 0040	10	0 0383	1 04	0 0091	
Gland, Prostate	ABS	10	0 7215	R REF	0 1036	10	0 7324	1 02	0 2129	
	OW:BW	10	0 2439	R REF	0 0328	10	0 2699	1 11	0 0726	
	OW:BRN	10	0 3781	R REF	0 0476	10	0 3808	1 01	0 0941	
Heart	ABS	10	0 9152	R REF	0 0698	10	0 9242	1 01	0 1151	
	OW:BW	10	0 3097	R REF	0 0260	10	0 3405	1 10	0 0329	*
	OW:BRN	10	0 4807	R REF	0 0388	10	0 4852	1 01	0 0758	
Kidney	ABS	10	2 1659	R REF	0 1836	10	2 2197	1 02	0 2229	
	OW:BW	10	0 7312	R REF	0 0411	10	0 8179	1 12	0 0507	†
	OW:BRN	10	1 1356	R REF	0 0682	10	1 1600	1 02	0 0939	
Liver	ABS	10	8 3218	R REF	0 5205	10	7 7880	0 94	0 4860	*
	OW:BW	10	2 8131	R REF	0 1435	10	2 8771	1 02	0 1801	
	OW:BRN	10	4 3681	R REF	0 2325	10	4 0850	0 94	0 3960	
Spleen	ABS	10	0 5951	R REF	0 0613	10	0 7700	1 29	0 1038	†
	OW:BW	10	0 2008	R REF	0 0147	10	0 2842	1 42	0 0352	†
	OW:BRN	10	0 3120	R REF	0 0264	10	0 4019	1 29	0 0431	†
Testis	ABS	**N**	**Mean**	**Ratio**	**SD**	**N**	**Mean**	**Ratio**	**SD**	
		10	3 2727	R REF	0 3106	10	3 4683	1 06	0 3109	
	OW:BW	10	1 1090	R REF	0 1254	10	1 2803	1 15	0 1001	*
	OW:BRN	10	1 7171	R REF	0 1440	10	1 8123	1 06	0 1262	
Thymus	ABS	10	0 5914	R REF	0 0676	10	0 4673	0 79	0 0934	†
	OW:BW	10	0 1999	R REF	0 0222	10	0 1718	0 86	0 0293	*
	OW:BRN	10	0 3098	R REF	0 0266	10	0 2448	0 79	0 0507	†

(b) (4)

Table 50: Male's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at the end of the terminal phase.

Body weight was (b) (4) . Spleen weight was increased 29% (b) (4) in groups (b) (4) , respectively. Thymus weight was decreased 21% (b) (4) in (b) (4) , respectively.

Table of organ weights results for females

Group Number:		REF				2				3	
Dose:		0 µg/day				30 µg/day				(b) (4)	
		N	Mean	Ratio	SD	N	Mean	Ratio	SD		
BWT	ABS	10	198 73	R REF	10 80	10	194 56	0 98	10 69		
Brain	ABS	10	1 8610	R REF	0 0694	10	1 7868	0 96	0 0595		
	OW:BW	10	0 9383	R REF	0 0507	10	0 9203	0 98	0 0467		
	OW:BRN	10	1 0000	R REF	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		
	OW:BW	10	0 0442	R REF	0 0068	10	0 0454	1 03	0 0065		
	OW:BRN	10	0 0474	R REF	0 0088	10	0 0496	1 05	0 0085		
Heart	ABS	10	0 7450	R REF	0 0803	10	0 7573	1 02	0 0866		
	OW:BW	10	0 3749	R REF	0 0343	10	0 3893	1 04	0 0417		
	OW:BRN	10	0 4004	R REF	0 0418	10	0 4248	1 06	0 0563		
Kidney	ABS	10	1 5273	R REF	0 0808	10	1 6343	1 07	0 0778	*	
	OW:BW	10	0 7696	R REF	0 0415	10	0 8412	1 09	0 0418	†	
	OW:BRN	10	0 8216	R REF	0 0519	10	0 9153	1 11	0 0477	†	
Liver	ABS	10	5 4571	R REF	0 3313	10	5 6490	1 04	0 5559		
	OW:BW	10	2 7466	R REF	0 0920	10	2 9002	1 06	0 1853	*	
	OW:BRN	10	2 9329	R REF	0 1468	10	3 1630	1 08	0 3132		
Ovary	ABS	10	0 1167	R REF	0 0158	10	0 1053	0 90	0 0180		
	OW:BW	10	0 0588	R REF	0 0076	10	0 0542	0 92	0 0097		
	OW:BRN	10	0 0627	R REF	0 0079	10	0 0590	0 94	0 0101		
Spleen	ABS	10	0 4382	R REF	0 0669	10	0 6796	1 55	0 1031	†	
	OW:BW	10	0 2202	R REF	0 0294	10	0 3492	1 59	0 0489	†	
	OW:BRN	10	0 2353	R REF	0 0333	10	0 3803	1 62	0 0550	†	
Thymus	ABS	10	0 4588	R REF	0 0700	10	0 3967	0 86	0 1131		
	OW:BW	10	0 2310	R REF	0 0336	10	0 2031	0 88	0 0583		
	OW:BRN	10	0 2469	R REF	0 0386	10	0 2221	0 90	0 0655		

(b) (4)

Table 51: Female's organ weight: Absolute weights are expressed as mean (grams). Entries in table are expressed as organ weight from animals taken at the end of the terminal phase.

Spleen weight was increased 55% (b) (4) in groups (b) (4), respectively. Thymus weight was decreased 14% and (b) (4) in groups (b) (4), respectively.

Gross pathology:

Dosing phase

In groups (b) (4), large draining lymph nodes (abnormal size, enlarged) and dark/pale and/or firm injection sites (abnormal color, dark/pale and/or abnormal consistency, firm) were reported. In group (b) (4), large spleen and inguinal lymph nodes (abnormal size, enlarged) were reported.

	Male			Female		
Group Number:	1	2	3	1	2	3
Dose:	0 µg/day	30 µg/day	(b) (4) /day	0 µg/day	30 µg/day	(b) (4) /day
Animals Examined:	10	10	10	10	10	10
LIVER			(b) (4)			(b) (4)
Abnormal surface	-	1		-	-	
LUNG						
Abnormal color	1	1		-	-	
LYMPH NODE, DRAINING						
Abnormal size	-	1		-	1	
LYMPH NODE, INGUINAL						
Abnormal size	1	-		-	-	
SITE, INJECTION						
Abnormal color	-	2		1	3	
Abnormal consistency	-	2		-	4	
SPLEEN						
Abnormal size	-	-		-	-	

Table 52: Gross findings at dosing phase

Recovery phase

In one group (b) (4), large draining lymph nodes (abnormal size, enlarged) were reported. Large inguinal lymph nodes (abnormal size, enlarged) were reported in one group (b) (4), indicating a partial recovery of these findings. In groups (b) (4), pale/dark and/or firm injection sites and enlarged spleen were not reported at the end of recovery phase, indicating a complete recovery of these findings.

	Male			Female		
Group Number:	1	2	3	1	2	3
Dose:	0 µg/day	30 µg/day	(b) (4)	0 µg/day	30 µg/day	(b) (4)
Animals Examined:	5	5		5	5	
LYMPH NODE, DRAINING						
Abnormal size	-	1		-	-	
LYMPH NODE, INGUINAL						
Abnormal size	-	-		-	-	
ADIPOSE TISSUE						
Abnormal color	1	-		-	1	
Abnormal consistency	1	-		-	-	

Table 53: Macroscopic findings at recovery phase

Microscopic findings:

Terminal sacrifice

In (b) (4), findings at 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) were reported.

	Group Number:	Male 1	2	3	Female 1	2	3
	Dose:	0 µg/day	30 µg/day	(b) (4) /day	0 µg/day	30 µg/day	(b) (4) /day
	No. Animals Per Dose Group:	10	10	10	10	10	10
EYE	Number Examined	10	10	(b) (4)	10	10	(b) (4)
	Unremarkable	10	10		9	9	
Mineralization, Cornea		-	-		-	1	
	Minimal	-	-		-	1	
Rosettes retina		-	-		1	-	
	Minimal	-	-		1	-	
GLAND, ADRENAL	Number Examined	10	10		10	10	
	Unremarkable	10	10		10	10	
Hypertrophy, Cortex		-	-		-	-	
	Present	-	-		-	-	
GLAND, HARDERIAN	Number Examined	10	10		10	10	
	Unremarkable	10	10		6	9	
Degeneration/Necrosis		-	-		2	-	
	Minimal	-	-		2	-	
Infiltration mononuclear cell		-	-		3	1	
	Minimal	-	-		3	1	
GLAND, PITUITARY	Number Examined	10	10		10	10	
	Unremarkable	10	9		9	10	
Cyst		-	1		1	-	
	Minimal	-	1		1	-	
GLAND, PROSTATE	Number Examined	10	10		-	-	
	Unremarkable	10	10		-	-	
Infiltration mononuclear cell		-	-		-	-	
	Minimal	-	-		-	-	

	Group Number:	Male 1	2	3	Female 1	2	3
	Dose:	0 µg/day	30 µg/day	(b) (4) /day	0 µg/day	30 µg/day	(b) (4) /day
	No. Animals Per Dose Group:	10	10	10	10	10	10
GLAND, SALIVARY	Number Examined	10	10	(b) (4)	10	10	(b) (4)
	Unremarkable	10	10		9	10	
Hypertrophy		-	-		1	-	
	Minimal	-	-		1	-	
GUT-ASSOCIATED LYMPHOID TISSUE	Number Examined	10	10		8	10	
	Unremarkable	10	10		8	9	
Mineralization, Germinal center		-	-		-	1	
	Minimal	-	-		-	1	
HEART	Number Examined	10	10		10	10	
	Unremarkable	10	10		10	10	
JOINT	Number Examined	10	10		10	10	
	Unremarkable	10	7		9	8	
Inflammation, Extra-capsular		-	3		-	2	
	Minimal	-	3		-	2	
Physeal dysplasia		-	-		1	-	
	Minimal	-	-		1	-	
KIDNEY	Number Examined	10	10		10	10	
	Unremarkable	9	9		8	6	
Tubular basophilia		-	1		-	1	
	Minimal	-	1		-	1	
Infiltration mononuclear cell		-	-		2	3	
	Minimal	-	-		2	3	
Dilatation, Pelvis		1	-		-	-	
	Minimal	1	-		-	-	

	Group Number:	Male 1	2	3	Female 1	2	3
	Dose:	0 µg/day	30 µg/day	(b) (4) /day	0 µg/day	30 µg/day	(b) (4) /day
	No. Animals Per Dose Group:	10	10	10	10	10	10
LARGE INTESTINE, COLON	Number Examined	10	10	(b) (4)	10	10	(b) (4)
	Unremarkable	10	10		10	10	
Infiltration mixed cell, Mucosa		-	-		-	-	
	Minimal	-	-		-	-	
LIVER	Number Examined	10	10		10	10	
	Unremarkable	10	5		10	0	
Vacuolation, Hepatocyte; Periportal		-	5		-	10	
	Minimal	-	5		-	10	
LUNG	Number Examined	10	10		10	10	
	Unremarkable	10	10		9	9	
Infiltration mixed cell		-	-		1	1	
	Minimal	-	-		1	1	
LYMPH NODE, DRAINING	Number Examined	10	9		10	10	
	Unremarkable	8	1		8	1	
Increased cellularity, Plasma cell		-	7		-	9	
	Minimal	-	1		-	1	
	Mild	-	4		-	1	
	Moderate	-	2		-	7	
Increased cellularity, Germinal center		2	6		2	5	
	Minimal	1	2		1	3	
	Mild	1	4		1	2	
LYMPH NODE, INGUINAL	Number Examined	9	10		10	10	
	Unremarkable	8	5		9	4	
Increased cellularity, Germinal center		1	5		1	6	
	Minimal	-	1		1	3	
	Mild	1	4		-	3	
Increased cellularity, Plasma cell		-	1		-	2	
	Minimal	-	1		-	2	

	Group Number:	Male 1	2	3	Female 1	2	3
	Dose:	0 µg/day	30 µg/day	(b) (4) /day	0 µg/day	30 µg/day	(b) (4) /day
	No. Animals Per Dose Group:	10	10	10	10	10	10
PANCREAS	Number Examined	10	10	(b) (4)	10	10	(b) (4)
	Unremarkable	10	10		10	6	
Atrophy, Acinar cell		-	-		-	4	
	Minimal	-	-		-	4	
Infiltration mononuclear cell, Interstitium		-	-		-	1	
	Minimal	-	-		-	1	
SITE, INJECTION	Number Examined	10	10		10	10	
	Unremarkable	6	0		5	0	
Inflammation		4	10		5	10	
	Minimal	4	-		5	-	
	Mild	-	7		-	7	
	Moderate	-	3		-	3	
Edema		-	9		-	10	
	Mild	-	8		-	9	
	Moderate	-	1		-	1	
SPLEEN	Number Examined	10	10		10	10	
	Unremarkable	10	0		10	0	
Increased cellularity, Germinal center		-	5		-	6	
	Minimal	-	5		-	6	
Increased cellularity, Hematopoietic cell		-	10		-	9	
	Minimal	-	10		-	9	
STOMACH	Number Examined	10	10		10	10	
	Unremarkable	10	10		10	9	
Infiltration mononuclear cell, Serosa		-	-		-	1	
	Minimal	-	-		-	1	
Erosion		-	-		-	-	
	Minimal	-	-		-	-	

Table 54: Microscopic findings at terminal sacrifice

Recovery sacrifice
A complete recovery of most of the findings reported at the terminal phase. Inflammation at the injection site was characterized by mostly lymphocytes and plasma cells with few neutrophils (indicating partial recovery) and no edema (full recovery). In (b) (4), 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. At the end of recovery phase, mature plasma cells had replaced the plasma blasts identified in the inguinal and draining lymph nodes in the dosing phase animals. Infiltration of macrophages was reported in the draining lymph nodes (minimal to mild) in (b) (4) and in the inguinal lymph nodes (minimal) of group 2 males and females.

Dermal Assessment

Dosing phase
In all group 2 (except animal #17) animals, related injection site edema grade 2 (slight, edges of area well defined by definite raising) or grade 3 (moderate, raised approximately 1 mm) were reported following dosing on days 1, 8 and/or 15. The edema was generally reported up to 72 hours post dose, and fully resolved prior to dose administration on days 8 and 15. In all group 2 (except animals 16-21 and 30) animals, erythema was also reported at the injection site, following each dose administration. However, it was only a grade 1 (very slight, barely perceptible) and fully resolved prior to the next dose administration.

In all group 3 (b) (4)

Group mean dermal assessment data are listed in the table below:

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	(b) (4)		
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.19	0.51	0.001 **
			3: BNT162b3c	15	(b) (4)		
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.33	0.45	0.001 **
			3: BNT162b3c	15	(b) (4)		

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	(b) (4)		
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.23	0.27	0.001 **
			3: BNT162b3c	15	(b) (4)		
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.00	0.00	0.999
			3: BNT162b3c	15	(b) (4)		

Table 55: Edema and erythema findings in males at study days 1, 8, and 15

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	(b) (4)		
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.44	0.23	0.001 **
			3: BNT162b3c	15	(b) (4)		
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	1.64	0.34	0.001 **
			3: BNT162b3c	15	(b) (4)		

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	(b) (4)		
	Dosing	8	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.50	0.09	0.001 **
			3: BNT162b3c	15	(b) (4)		
	Dosing	15	1: Saline	15	0.00	0.00	REF
			2: BNT162b2 (V9)	15	0.33	0.22	0.001 **
			3: BNT162b3c	15	(b) (4)		

Table 56: Edema and erythema findings in females at study days 1, 8, and 15

Male

Parameter	Phase	Group	N	Mean	Standard Deviation
Edema - Left	Recovery	2: BNT162b2 (V9)	4	1.08	0.17
		3: BNT162b3c	5	(b) (4)	
Erythema - Left	Recovery	2: BNT162b2 (V9)	4	0.00	0.00
		3: BNT162b3c	5	(b) (4)	

Female

Parameter	Phase	Group	N	Mean	Standard Deviation
Edema - Left	Recovery	2: BNT162b2 (V9)	5	1.07	0.15
		3: BNT162b3c	5	(b) (4)	
Erythema - Left	Recovery	2: BNT162b2 (V9)	5	0.13	0.18
		3: BNT162b3c	5	(b) (4)	

Table 57: Edema and erythema findings in males and females at recovery phase

Body temperature:
No test article-related effects on body temperature was reported.

Urinalysis:
There were no test article-related findings on urinalysis. Due to small magnitude of the difference and general overlap in magnitude of individual values with controls, all statistically significant or apparent differences in urinalysis parameters between groups 2 and 3 and control group were not test article related.

			Male							
			Group Number:	REF		2			3	
	Phase	Day	Dose :	0 µg/day		30 µg/day			(b) (4)	
pH (None)	Dosing	17	Mean	(10)	7.10	(10)	6.75	(10)		†
			SD		0.39		0.35			
	Recovery	22	Mean	(5)	7.30	(5)	7.20	(5)		
			SD		0.45		0.27			
SG (None)	Dosing	17	Mean	(10)	1.0322	(10)	1.0260	(10)		
			SD		0.0205		0.0227			
	Recovery	22	Mean	(5)	1.0556	(5)	1.0340 *	(5)		
			SD		0.0038		0.0146			
VOLUME (mL)	Dosing	17	Mean	(10)	14.90	(10)	17.80	(10)		
			SD		15.54		16.95			
	Recovery	22	Mean	(5)	3.70	(5)	8.20	(5)		
			SD		0.97		5.50			

Table 58: Urinalysis for male groups

			Female							
			Group Number:	REF		2		3		
	Phase	Day	Dose :	0 µg/day		30 µg/day		(b) (4)		
pH (None)	Dosing	17	Mean	(10)	6.75	(10)	6.20 †	(10)		†
			SD		0.26		0.26			
	Recovery	22	Mean	(5)	7.00	(5)	6.60	(5)		
			SD		0.61		0.65			
SG (None)	Dosing	17	Mean	(10)	1.0243	(10)	1.0288	(10)		
			SD		0.0128		0.0164			
	Recovery	22	Mean	(5)	1.0240	(5)	1.0364	(5)		
			SD		0.0174		0.0177			
VOLUME (mL)	Dosing	17	Mean	(10)	9.90	(10)	9.60	(10)		
			SD		7.03		9.05			
	Recovery	22	Mean	(5)	11.00	(5)	6.00	(5)		
			SD		7.38		5.09			

Table 59: Urinalysis for male groups

Serology:

Microneutralization (MN) assay for serological detection of SARS-CoV-2 specific neutralizing antibodies in animal sera were used. This is relative to the "work order 4" agreed between (b) (4) and Pfizer. The (b) (4) method is a specific technique used for the (b) (4) . The following table shows geometric mean titers for grouped subjects by sex and for vaccine administered.

Study Day	Sex	saline	30µg BNT162b2(V9)	(b) (4) BNT162b3c
PIO Day 8 (Day -5)	Male	5	5	(b) (4)
	Female	5	5	
Day 17	Male	5	1114	
	Female	5	2501	
R:P Day 21 (Day 38)	Male	5	5120	
	Female	5	5120	
PIO = prior to dose initiation; RP = Recovery phase				

Table 60: Geometric mean titers (GMTs) for each dose group by sampling day and sex

In (b) (4) , SARS-CoV-2 neutralizing antibody responses in males and females at the end of the dosing (day 17) and recovery phases (day 21) were reported. SARS-CoV-2 neutralizing antibody responses were not reported in animals prior to vaccine administration or in group 1 (control) animals.

Test article related effects are listed in the table below:

Test article related effects
↓ Albumin ↑ Globulin ↓ AG ratio

Test article related effects
↓ Reticulocytes
↑ Monocytes
↑ Neutrophils
↑ Eosinophils
↑ Basophils
↑ WBC
↑ LUC
↑ Fibrinogens
↑ Red cell distribution width (RDW%)
↑ Alpha1-acid glycoproteins
↑ Alpha2-macroglobulins
↑ Spleen weight
↓ Thymus weight for females
Injection site findings (mixed cell inflammation and edema)
Draining and inguinal lymph nodes findings (increased cellularity, plasma cells and germinal centers)
Liver findings (hepatocellular vacuolation)
Spleen findings (increased cellularity, hematopoietic cells and germinal centers)
Bone marrow (increased cellularity, hematopoietic cells)
Immune responses in (b) (4)

Assessment:
No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight, food consumption, body temperature, ophthalmic changes, or urinalysis were reported.

Minimal decreases in globulin concentration was reported in (b) (4). Concurrently, minimally increased albumin was reported. Hence, the albumin to globulin ratio was lower in (b) (4) from (b) (4). These changes indicate an acute phase response/inflammation. These changes were not reported in the recovery animals.

Reticulocytes are immature red blood cells (RBCs). In the process of erythropoiesis (red blood cell formation), reticulocytes develop and mature in the bone marrow and then circulate for about a day in the blood stream before developing into mature red blood cells. Like mature red blood cells, in mammals, reticulocytes do not have a cell nucleus.[26] Abnormally low numbers of reticulocytes can be attributed to chemotherapy, aplastic anemia, pernicious anemia, bone marrow malignancies, problems of erythropoietin production, various vitamin or mineral deficiencies (iron, vitamin B_{12}, folic acid), disease states (anemia of chronic disease) and other causes of anemia due to poor RBC production.[27]

Monocytosis could be indicative of the intended immune response or could be secondary to muscle damage at the site of injection as an indication of inflammation and repair. The increases in the monocyte count might be related to test article treatment.

[26] https://en.wikipedia.org/wiki/Reticulocyte
[27] https://www.uofmhealth.org/health-library/hw203366

Neutrophils are key components in the system of defense against infection. An individual with absence or scarcity of neutrophils (neutropenia) is vulnerable to infection. The increase in neutrophils might be related to the immune responses initiated by the test article treatment.

Eosinophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood.

Basophils play a role in both parasitic infections and allergies. Basopenia has been reported in association with autoimmune urticaria.

White blood cells (WBCs) (also called leukocytes or leucocytes) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.[28] The increase in WBC might be related to the immune response induced by the test article treatment.

LUC is a measurement of the large, peroxidase-negative cells which cannot be further characterized (i.e. as large lymphocytes, virocytes, or stem cells) present in a biological specimen. In LUC are found large lymphoid cells, more immature lymphocytes and other cells. If the value is higher than normal, blood counts should be checked under a microscope slide.

The increases in fibrinogen levels were not considered frank toxicity but rather an anticipated effect associated with an immunological response.

A red cell distribution width (RDW) test is a measurement of the range in the volume and size of red blood cells (erythrocytes). Red blood cells move oxygen from lungs to every cell in the body. The RDW blood test is often part of a complete blood count (CBC), a test that measures many different components of the blood, including red cells. The RDW test is commonly used to diagnose anemia, a condition in which the red blood cells can't carry enough oxygen to the rest of the body. The RDW test may also be used to diagnose[29]:

1- Other blood disorders such as thalassemia, an inherited disease that can cause severe anemia
2- Medical conditions such as heart disease, diabetes, liver disease, and cancer, especially colorectal cancer.

Alpha-1-acid glycoprotein (*α_1AGp*,[30] *AGP* or *AAG*), which is modulated by two polymorphic genes, is an acute phase (acute phase protein) plasma alpha-globulin glycoprotein. It has a

[28] *Maton, D., Hopkins, J., McLaughlin, Ch. W., Johnson, S., Warner, M. Q., LaHart, D., & Wright, J. D., Deep V. Kulkarni (1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall. ISBN 0-13-981176-1.*
[29] https://medlineplus.gov/lab-tests/rdw-red-cell-distribution-width/
[30] https://en.wikipedia.org/wiki/Orosomucoid#cite_note-loganabbrev-1

normal plasma concentration between 0.6-1.2 mg/mL (1-3% plasma protein) and is synthesized primarily in hepatocytes (5). Plasma levels are affected by pregnancy, burns, certain drugs, and certain diseases, particularly HIV (5). The function of alpha-1-acid glycoprotein is to act as a carrier of basic and neutrally charged lipophilic compounds. It is known as the primary carrier of basic (positively charged) drugs (whereas albumin carries acidic (negatively charged) and neutral drugs), steroids, and protease inhibitors (5, 6). AGP shows a complex interaction with thyroid homeostasis. Alpha-1-acid glycoprotein (in low concentrations) was reported to stimulate the thyrotropin (TSH) receptor and intracellular accumulation of cyclic AMP. However, high AGP concentrations inhibited TSH signaling (7, 8). Alpha-1-acid glycoprotein has been identified as one of four potentially useful circulating biomarkers for estimating the five-year risk of all-cause mortality (the other three are albumin, very low-density lipoprotein particle size, and citrate) (9). Alpha-1-acid glycoprotein increases in obstructive jaundices while diminishes in hepatocellular jaundice and in intestinal infections.[31]

Alpha-2-macroglobulin (α2M) is a large plasma protein found in the blood, mainly produced by the liver, and also locally synthesized by macrophages, fibroblasts, and adrenocortical cells. It acts as an antiprotease and is able to inactivate an enormous variety of proteinases. It functions as an inhibitor of fibrinolysis by inhibiting plasmin and kallikrein and as an inhibitor of coagulation by inhibiting thrombin. Because it also binds to numerous growth factors and cytokines, such as platelet-derived growth factor, basic fibroblast growth factor, TGF-β, insulin, and IL-1β, it may act as a carrier protein. In the nephrotic syndrome when other lower molecular weight proteins are lost in the urine, the concentration of alpha-2-macroglobulin rises 10-fold or more[32].

In (b) (4) all clinical pathology findings (type and magnitude) were generally similar, and consistent with expected immune responses to vaccines or secondary to inflammation. In both sexes, the main findings were present 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). On peripheral blood smears, hyper-segmented neutrophils present and 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 (10). These findings were consistent with the immune responses to vaccines.

Spleen weight increase might be related to the intended immune response. The spleen plays important roles in regard to red blood cells and the immune system[33]. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes. The globin portion of hemoglobin is degraded to its constitutive amino acids, and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for

[31] https://en.wikipedia.org/wiki/Orosomucoid
[32] https://en.wikipedia.org/wiki/Alpha-2-Macroglobulin
[33] Spleen, Internet Encyclopedia of Science.

removal[34]. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

The thymus is a specialized primary lymphoid organ of the immune system. Within the thymus, T cells or T lymphocytes mature. T cells are critical to the adaptive immune system, where the body adapts specifically to foreign invaders. The thymus is composed of two identical lobes and is located anatomically in the anterior superior mediastinum, in front of the heart and behind the sternum.[35] One of the major characteristics of vertebrate immunology is thymic involution, the shrinking of the thymus with age, resulting in changes in the architecture of the thymus and a decrease in tissue mass.[36] T-cells are named for the thymus where T-lymphocytes migrate from the bone marrow to mature. Its regression has been linked to the reduction in immunosurveillance in the elderly.[37]

Test article-related injection site findings (mixed cell inflammation and edema) were reported. Inflammation is a relatively common occurrence as part of the acute phase response following administration of some vaccines.

The microscopic findings 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, 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) were reported. Lower reticulocytes levels were interpreted to be a transient effect of innate immune responses (11-14).

At the terminal 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 reported. This is considered secondary to immune activation and/or inflammation at the injection site. The presence of plasma cells (interpreted as plasma blasts) in the draining and inguinal lymph nodes was interpreted to reflect a robust immunological response to the vaccines. These findings correlated with macroscopic findings of abnormal size (enlarged) in the lymph nodes and spleen and increased spleen weights.

Minimal portal hepatocyte vacuolation finding 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 (15). This finding was completely recovered at the end of 3-week recovery phase.

Test article-related immune responses in groups 2 and 3 were reported.

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[34] Mebius RE, Kraal G. (2005). Structure and function of the spleen. Nat Rev Immunol. 5(8):606-16.
[35] https://en.wikipedia.org/wiki/Thymus.
[36] *Shanley D.P.; Danielle A.W.; Manley N.R.; Palmer D.B.; et al. (2009). "An evolutionary perspective on the mechanisms of immunosenescence". Trends Immunol.* ***30*** *(7): 374–381.* doi:10.1016/j.it.2009.05.001. PMID 19541538
[37] *Linton P.J.; Dorshkind K. (2004). "Age-related changes in lymphocyte development and function". Nat. Immunol.* ***5*** *(2): 133–139.* doi:10.1038/ni1033. PMID 14749784

Based on the overall findings in this study, it can be concluded that in Wistar rats, repeat dose on study days 1, 8, and 15 had no adverse effects in terms of systemic toxicity at the dose level of 30 µg/animal. However, due to the significant decrease in the reticulocyte levels, hematology results should be closely monitored during any clinical trial.

GLP study deviations or amendments: No significant deviations have occurred during the study that could have impacted the generated results.

Investigators Brochure: Having read and evaluated the Investigators Brochure, is it a fair, objective and reasonable summary of the toxicology data – yes () or no (X).

Internal Communication:
Due to the significant decreases in the reticulocyte's levels, close monitoring to the hematology data in any clinical trial is highly recommended.

Communication to sponsor:
Please add the finding of this study to your Investigators Brochure.

Conclusions:
Based on nonclinical toxicity assessments, there are no significant safety issues to preclude the IND from going into effect.

Study number 3 (Reproductive Toxicology Study):

Title and study number: A Combined Fertility and Developmental Study (Including Teratogenicity and Postnatal Investigations) of BNT162b1, BNT162b2 and BNT162b3 by the Intramuscular Administration in the Wistar Rat. Study number: 20256434.

Performing laboratory: (b) (4)

Study initiation date: July 27, 2020

Final Report date: December 15, 2020

Test article batch/lot:

Test item identification

	Test Item 1	Test Item 2	Test Item 3
Identification:	BNT162b1	BNT162b2	BNT162b3
Alternate Identification:	(b) (4)	CoVVAC	(b) (4)
Batch No.:		(b) (4)	
Lot No.:		CoVVAC/270320	
Physical Description:		White to off-white suspension	
Expiry Date:		27 Nov 2020	
Correction Factor:		None	
Concentration (RNA Content):		(b) (4)	
Storage Conditions:	Temperature set to maintain -80°C		
Provided by:	Sponsor		

Table 61: Test item identification

Control item identification

	Control Item
Identification:	Sterile physiological saline (0.9% NaCl)
Alternate Identification:	N/A
Batch/Lot Nos.:	(b) (4)
Expiry Dates:	30 Apr 2022 and 30 Nov 2022 respectively
Storage Conditions:	Ambient temperature
Provided by:	Test Facility

N/A: Not Applicable.

Table 62: Control item identification

Animal species and strain: (b) (4):WI(Han) Wistar rat

Breeder/supplier: (b) (4)

Number of animals per group and sex:

Caesarean subgroup: 88 virgin mated females.

Littering subgroup: 88 virgin mated females.

Age:

Females: 11 weeks old.
Males: 11 weeks old.

Body weight range:

Females: 179.3 - 265.4 g.
Males: 328.4 - 415.9 g.

Route and site of administration: Intramuscular injection into the quadriceps alternating on each dosing occasion.

Volume of injection: The dose volume was 0.06 mL per injection

Frequency of administration and study duration:

Pre-mating period: Study days 1 (21 days before mating, M-21) and 8 (14 days before mating, M-14) and on gestation days (GD's) 9 and 20.

Dose: 0.5 mg/mL

Stability: Analysis of stability, homogeneity and concentration of the test article under test conditions was not performed as part of the study. Stability studies were performed by the sponsor of the IND. Stability data will be reported in the final study report. The following stability data were reported:

1- Stable at a concentration of 0.5 mg/mL for 12 weeks at -80°C.
2- Stable at a concentration of 0.5 mg/mL for at least 1 month at room temperature (information provided by the study sponsor on 03 Dec 2020)
3- Homogenous for at least 6 hours following gentle inversion.

Means of administration: Intramuscular

Report status: Final report

Experimental design:

Animals were randomized and assigned to 4 different groups. Each group consisted of 22 females. Animals were administered 4 doses of saline or test article, study day 1 (21 days before mating, M-21) and day 8 (14 days before mating, M-14) and on gestation days 9 and 20. Animals will be euthanized according to the following schedule:

F0 Females: Caesarean subset: On GD21.
Littering subset: After weaning of the F1 pups (females that fail to produce a viable litter by GD26 will be euthanized and necropsied).
Unmated Females: After completion of the mating period.
Pups: On PND4 (unselected pups) or on PND21.

The details of the study design are listed in the following table:

Experimental design of the F0 generation

Group No.	Test Material	Dose (µg mRNA)	Dose Volume (mL)	Dose Concentration (mg/mL)	Number and Identification of Animals	
					Caesarean Subgroup	Littering Subgroup
1	Control item	0	0.06	0	22 (1 to 22)	22 (201 to 222)

Group No.	Test Material	Dose (µg mRNA)	Dose Volume (mL)	Dose Concentration (mg/mL)	Number and Identification of Animals	
					Caesarean Subgroup	Littering Subgroup
2	BNT162b1	(b) (4)				
3	BNT162b2	30	0.06	0.5	22 (45 to 66)	22 (245 to 266)
4	BNT162b3	(b) (4)				

(b) (4)

Identification of untreated males: 301 to 388.

Table 63: Experimental design of the F0 generation

Methods:
Randomization procedure: Yes.
Statistical analysis plan: Yes.

The following parameters will be evaluated:
In-life procedures, observations, and measurements
General in-life assessments – untreated males and F0 females

Parameter	Population(s)	Frequency (Minimum required)	Comments
Mortality	All animals	At least twice daily[a] (at beginning and end of working day) F1 pups will be counted daily during the preweaning phase	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings
Cageside Observations	All animals	Before and at least once on dosing days For males, at least 1 observation will be recorded before mating At least once daily on non-dosing days	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings
Detailed Clinical Observations	All animals	A full clinical examination will be performed weekly during the pre-mating period then on each weighing day during the gestation and lactation periods	Animals will be removed from the cage
Individual Body Weights	All F0 females	Each F0 female will be weighed at least weekly during pretest, **twice weekly** before mating and for the periods: GD0, GD6, GD9, GD12, GD15, GD18 and GD21 LD1, LD4, LD7, LD10, LD14, LD17 and LD21 During the lactation phase, offspring were weighed on PND1, PND4, PND7, PND10, PND14, PND17 and PND21.	Animals may be weighed more often if necessary, in order to monitor health status

Parameter	Population(s)	Frequency (Minimum required)	Comments
	All F0 males	Each F0 male will be weighed at least weekly	
Food Consumption	All F0 females	Food consumption of each animal will be recorded at least **once weekly** from Day 1 and for the periods: GD0 to GD6, GD6 to GD9, GD9 to GD12, GD12 to GD15, GD15 to GD18 and GD18 to GD21 LD1 to LD4, LD4 to LD7, LD7 to LD10, LD10 to LD14, LD14 to LD17 and LD17 to LD21	Quantitatively measured
Estrous Cycles	All F0 Females	Estrous cycles will be monitored pre-dosing (2 weeks), then for 2 weeks before mating and during cohabitation until confirmation of GD0	Animals are removed from the cage
Mating	Males and all F0 Females	Animals will be paired on the basis of 1 male and 1 female for a maximum of 14 days The day of mating will be confirmed by the presence of sperm in a vaginal smear or a vaginal plug and will be recorded and taken as day 0 of gestation (GD0) **The same untreated males will be used to mate both subgroups**	Mated females will be separated from the male once mating has been confirmed and smearing will cease or when the appearance of the female suggests pregnancy from an undetected mating

[a]: Except on days of receipt and necropsy where frequency will be at least once daily.

Table 64: General in-life assessments – untreated males and F0 females

Pregnancy and parturition (littering subset only)

For each F0 female, the following will be recorded:

1- Date of mating (GD0).
2- Date of parturition (LD0).
3- Duration of gestation.
4- Abnormalities of nesting or nursing behavior.
5- Number of implantation sites (at necropsy after staining with ammonium sulphide solution).

Litter data (littering subset only)

Litter data

Population(s)	Frequency/Comments
Each Litter	Number of pups born (live and dead)
	External abnormalities of the pups
	Number and sex of pups alive on PND1, PND4, PND7, PND10, PND14, PND17 and PND21
	Physical development of the offspring, as assessed by the intra-litter onset and duration of pinna unfolding from PND1 and eye opening from PND12. Pupillary reflex and auditory reflex on PND21.
	External and necropsy findings of dead pups

The size of each litter will be adjusted to 8 pups on PND4 by eliminating extra pups by random selection to yield where possible 4 male and 4 female pups per litter. Extra pups will be euthanized by an intraperitoneal injection of sodium pentobarbitone.

Antibody evaluation

Antibody sample collection

Bioanalytical sample collection

Group Nos.	Number of Females	Pre-dose on Days of Dosing		Necropsy (GD21 or LD21/PND21)[b]
		Pretest	M0[a]	
1 to 4	All F0 females	X	X	X
1 to 4	Selected fetuses from all litters of caesarean subset	-	-	X
1 to 4	Selected pups (1 male and 1 female if possible) from all litters of the lactation subset	-	-	X
Unscheduled euthanasia (when possible, **done in the animal facility**)		X		

X: Sample collected; -: Not collected.
M0: First day of pairing; GD: Gestation day; LD: Lactation day; PND: Post natal day.
a: Sample collected just before pairing.
b: The day of necropsy (i.e., (b) (4)); on GD26 for not pregnant F254 (BNT162b2, 30 µg); (b) (4)

Method/Comments:	F0 females: Jugular vein Fetuses: Small incision after anesthesia Pups: Intracardiac
Target Volume (mL):	Target 0.5 mL for F0 females Target 0.3 mL pooled per litter for fetuses Targeted 0.5 mL pooled per litter from 2 pups (ideally 1 male and 1 female)
Anticoagulant:	None
Special Requirements:	None
Processing	Serum

Unscheduled necropsy

Animals	Examination
Not Mated Females	Full macroscopic examination of the thoracic and abdominal cavities, **including the injection sites**. Any abnormalities observed will be sampled and preserved
Mated Females	Full macroscopic examination of the thoracic and abdominal cavities, **including the injection sites**, to determine their pregnancy status, number of corpora lutea and numbers and types of uterine implantations Any abnormalities observed will be sampled and preserved. Any fetuses from these females will **not be examined and discarded**

Scheduled euthanasia

Surviving animals will euthanized by carbon dioxide inhalation and exsanguination (with the exception of the PND4 extra pups) and then necropsied according to the following schedule:

F0 females: Caesarean subgroup: On GD21.

F43 that failed to mate was euthanized after the mating period (on day 43).

Littering subgroup: On LD21, after weaning of the F1 pups.
F226 and F254 that failed to produce a viable litter by GD26 or GD27 were euthanized and necropsied; F277 with a mistimed pregnancy (mating not detected) was euthanized and necropsied after the end of the mating period on day 43).
Culled F1 pups: On PND4.
Euthanized F1 pups: On PND21.

Necropsy

Caesarean subset

All animals will be submitted to a full macroscopic examination of the abdominal and thoracic cavities including the injection sites. Any abnormalities observed will be recorded and preserved but not examined further in first instance. For each female euthanized on GD21, the ovaries and uterus will be removed and examined including examination of the placentae. The following data will be recorded:

Necropsy data

Parameters	Comments
Pregnancy status	-
Gravid uterus weight	The uterus of apparently non-pregnant females was placed in ammonium sulphide solution in order to stain any previously undetected implantation sites
Number and distribution of intrauterine implantations	Classified as: Live fetuses, dead fetuses, early resorptions and late resorptions
Number of corpora lutea	-
Fetal weights	Individual weights were recorded
Fetal sex	-

-: No comment.

Subgroup 2 (Natural delivery)

The carcasses of PND21 pups were preserved for possible skeletal examinations. No further examination was performed.

For all F0 females, the number of implantation sites were recorded.

Fetal examination

Each fetus was examined for external defects and euthanized by oral administration of sodium pentobarbitone. Approximately one half of each litter was submitted to fresh visceral examination of the body (abdominal and thoracic cavities). The head was fixed in Harrison's fluid for subsequent examination by serial sectioning. The remaining carcass was retained and fixed in ethanol.

The remaining half of the fetuses in each litter was eviscerated and then processed for skeletal examination. The skeletal examinations were performed following maceration of the soft tissues with aqueous potassium hydroxide, staining of the skeleton with Alizarin red then passage into glycerol. Soft tissue and skeletal examinations were performed using a binocular microscope.

Results:

Serum Antibody Analysis

In groups (b) (4), 3, (b) (4), administration of 4 doses (2 prior to mating and 2 during gestation) of the test article elicited SARS-CoV-2 neutralizing antibody responses in the majority of females just prior to mating (M-14), at the end of gestation (GD21), and at the end of lactation (LD21). In most offspring (fetuses on GD21 and pups on PND21), SARS-CoV-2 neutralizing titers were also detected. In animals prior to vaccine administration or in saline-administered control animals, SARS-CoV-2 neutralizing antibody titers were not reported.

The following table shows geometric mean titers (GMT) by time-point (Interval/Occasion) and by group of females or offspring (fetuses and pups).

Interval/Occasion	Saline	BNT162b1	BNT162b2	BNT162b3
Pretest	5.0	(b) (4)	5.3	(b) (4)
MO	5.0		3886.4	
GD21 (Dams)	5.0		3445.5	
LD21	5.0		3620.4	
Fetuses (GD21)	5.0		640.0	
Pups (PND21)	5.0		4561.4	

Time-point legend:
MO= just prior to mating
GD21 = gestation day 21
LD21= lactation day 21
PND21= post-natal days

These GMTs exclude values from no pregnant females and other intermittent sample time points .
See Appendix 1 footnotes for list of all excluded samples, in data table they are marked (*) .

Table 65: Geometric mean titer by time-point and by group of females or offspring (fetuses and pups)

Mortality

No test article-related death was reported. (b) (4) . One group 3 animal delivered 8 stillborn pups. All these findings were not different than that reported in the historical data. Such cases of total litter death at or shortly after birth are present in the historical control data (2 studies (A19 in 2019 and V17 in 2017) out of 18 between 2015 and 2019).

Clinical observations

No adverse clinical signs during the premating and gestations periods related to any of the 3 vaccine candidates were reported.

Swelling (associated or not with limping and/or piloerection for 1 or 2 days after the second dose only) was reported at the injection site of groups (b) (4), 3, (b) (4) animals on mating day 21 (M-21), M-14, gestation day 9 (GD9) and GD 20.

No adverse clinical signs during the lactation period related to any of the 3 vaccine candidates were reported.

Abnormal vocalization, chromodacryorrhea, desquamation, erythema, localized hair loss, malocclusion, long or missing teeth, red vaginal discharge, red stained fur, scab(s), sore(s) were reported sporadically across the groups. These findings were considered to be incidental, related to the method of dose administration or to the pregnancy status of the females.

Body weight and food consumption
No test article-related body weight changes or food consumption was reported.

In groups (b) (4), compared with the control group (33 g) throughout the lactation phase. This was not considered vaccine-related, but due to an atypical high value in the control group compared with the historical control data range (from (b) (4)).

Estrous Cycle Data
No test article-related effect on the estrous cycle was reported.

Parameter	Cycle length (days)	Irregularity index	Percentage of estrus days	Percentage of females acyclic or with acyclic period
Group 1, Control, 0 µg				
MEAN	4.02	0.19	26.95	
SD	0.19	0.30	6.14	0
N	44	44	44	
Group 2, BNT162b1, (b) (4)				
MEAN	(b) (4)			
SD				
N				
Group 3, BNT162b2, 30 µg				
MEAN	4.00	0.18	26.70	
SD	0.11	0.30	5.00	4.5
N	42	42	42	
Group 4, BNT162b3, (b) (4)				
MEAN	(b) (4)			
SD				
N				

Table 66: Mean estrous cycle data - Before dosing

Parameter	Cycle length (days)	Irregularity index	Percentage of estrus days	Percentage of females acyclic or with acyclic period
Group 1, Control, 0 µg				
MEAN	4.00	0.03	25.19	
SD	0.00	0.14	3.94	18.2
N	36	36	36	
Group 2, BNT162b1, (b) (4)				
MEAN	(b) (4)			
SD				
N				
Group 3, BNT162b2, 30 µg				
MEAN	4.02	0.05	24.07	
SD	0.13	0.12	3.66	18.2
N	36	36	36	
Group 4, BNT162b3, (b) (4)				
MEAN	(b) (4)			
SD				
N				

Table 67: Mean estrous cycle data - Pre-mating period

Maternal Mating Performance and Fertility

No test article-related effects on mating performance or fertility was reported.
In total (caesarean and littering subgroups combined), 44, (b) (4), 44 and (b) (4) (out of 44) females mated in groups 1, (b) (4), 3, and (b) (4), respectively (including F277, from group (b) (4), not detected at the time of mating).

Therefore, the copulation index was 100, (b) (4), 100, and (b) (4) in groups 1, (b) (4), 3, and (b) (4), respectively.

Mated females (majority) were inseminated within the first 4 days of pairing (approximate duration of a normal estrous cycle). The mean pre-coital interval was consequently 3.0, (b) (4), 2.8 and (b) (4) days in groups 1, (b) (4), 3, and (b) (4), respectively. In total, there were 43, 41, 42, and 44 pregnant females out of 44 per group paired in groups 1, (b) (4), 3, and (b) (4), respectively. Therefore, the pregnancy rate was 98%, (b) (4), 95% and (b) (4) in groups 1, (b) (4), 3, and (b) (4), respectively.
In total, there were 43/44, (b) (4), 42/44 and (b) (4) pregnant/mated females in groups 1, (b) (4), 3, and (b) (4), respectively. Therefore, the fertility index was 98%, 95%, (b) (4) and (b) (4) in groups 1, (b) (4), 3, and (b) (4), respectively.

GROUP	1	2	3	4
DOSING	Control 0 µg	BNT162b1 (b) (4)	BNT162b2 30 µg	BNT162b3 (b) (4)
LITTERING AND CAESAREAN SUBSETS:				
NUMBER OF FEMALES:		(b) (4)		(b) (4)
Paired	44		44	
Failed to mate	0		0	
Inseminated	44		44	
Not pregnant	1C		1C+1L	
Mistimed pregnancy	0		0	
Pregnant	43		42	
PRE - COITAL INTERVAL - DAYS				
MEAN	3.0		2.8	
SD	2.2		1.7	
N	44		44	
COPULATION INDEX (%)	100		100	
PREGNANCY RATE (%)	98		95	
FERTILITY INDEX (%)	98		95	
Caesarean phase (inseminated females)				
- With viable fetuses	21		21	
Lactation phase (inseminated females)				
- Females with live pups [2]	22		21	
- Euthanized moribund post-partum	0		0	
- Total litter death post-partum	0		0	
- Reared pups to weaning	22		21	
GESTATION INDEX (%)	100		100	

C: Caesarean phase
L: Lactation phase
[1] mistimed pregnancy for one pair of rats
[2] including one euthanized moribund post-partum female from group (b) (4)

Table 68: Summary of cohabitation data and maternal performance in littering and Caesarean subsets

Caesarean data

Gravid uterus weight

No test article-related effects on mean gravid uterus weight were reported.

Mean gravid uterus weight and maternal body weight change

Day(s): G21 Relative to Mating (Litter: A)

Sex: Female		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Gravid Uterus (g)	Mean	86.32 R,k[1]	(b) (4)	87.65	(b) (4)
	SD	7.69		13.48	
	N	21		21	
	%Diff	-		1.53	
Necropsy BW (g)	Mean	366.51 I,a[2]		351.47	
	SD	24.72		26.24	
	N	21		21	
	%Diff	.		-4.11	
Adjusted BW (g)	Mean	280.19 L[4]		263.82	
	SD	22.08		15.75	
	N	21		21	
	%Diff	.		-5.84	
Net BWC from G6 (g)	Mean	104.25 [3]		93.20 dd[6]	
	SD	7.27		15.12	
	N	21		21	
	%Diff	.		-10.61	
Net BWC - Uterine Wt (g)	Mean	17.93 [3]		5.55 ddd[7]	
	SD	7.54		8.56	
	N	21		21	
	%Diff	.		-69.06	
Mean Foetal Wt (Both) (g)	Mean	4.89 I[8]		4.90	
	SD	0.23		0.30	
	N	21		21	
	%Diff	.		0.25	
No. Live Foetuses	Mean	13.2 R,k[1]		13.1	
	SD	1.6		2.1	
	%Diff	.		-0.4	

+ [Footnote is displayed in the comments and markers page]

1 [R,k - Automatic transformation: Rank, (all groups) test: Kruskal-Wallis $p < 0.05$]

2 [d - Test: Dunnett Non-Parametric 2-sided $p < 0.05$]

3 [I,a - Automatic transformation: Identity (no transformation), (All groups) Test: Analysis of variance $p < 0.05$]

4 [L - Automatic transformation: Log]

5 [R,kkk - Automatic transformation: Rank, (all groups) Test: Kruskal-Wallis $p < 0.001$]

6 [dd - Test: Dunnett Non-Parametric 2-sided $p < 0.01$]

7 [ddd - Test: Dunnett Non-Parametric 2-sided $p < 0.001$]

8 [I,aaa - Automatic transformation: Identity (no transformation), (all groups) test: Analysis of variance $p < 0.001$]

9 [ddd - Test: Dunnett 2-sided $p < 0.0$ 1]

0 [d - Test: Dunnett 2-sided p < 0.05]
Table 69: Mean gravid uterus weight and maternal body weight change

Pregnancy incidence
No test article-related effects on pregnancy incidence were reported. At the terminal Caesarean examinations, there were 21/22, (b) (4), 21/22, and (b) (4) pregnant/mated females in groups 1, (b) (4), 3, and (b) (4), respectively. All of which had viable fetuses.

Pre-implantation data
No test article-related effects on the pre-implantation data were reported. The mean numbers of corpora lutea and implantation sites were comparable in all groups.

The mean percentage pre-implantation loss was higher in groups 3 (b) (4) (9.77% and (b) (4), respectively) compared with the control group (4.09%). However, the differences remained within the historical control data range ((b) (4)) for pivotal studies. Thus, the difference was considered to be incidental.

Post-implantation data
No test article-related effects on embryo-fetal survival were reported. The mean percentage post-implantation loss and the mean live litter size were comparable in all groups and consistent with the historical control data.

Fetal data
No test article-related effects on mean fetal weight or fetal sex ratio were reported.

Mean Caesarean section data

Sex: Female Day(s) Relative to Mating (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Females Pregnant [CHSQFS]	N+ve	21	(b) (4)	21	(b) (4)
Dams with Viable Foetuses		21		21	
No. of Corpora Lutea [GEN AN]	Mean	14.7 I[1]		15.5	
	SD	1.6		2.1	
	Sum	309 I[1]		326	
No. of Implantations [GEN AN]	Mean	14.1 R[2]		14.0	
	SD	1.6		2.2	
	Sum	296 R[2]		294	
Pre-Implantation Loss [GEN AN]	Mean	0.6 R,k[3]		1.5 d[4]	
	SD	1.0		1.3	
	Sum	13 R,k[3]		32 d[4]	
Pre-Implantation Loss (%) [KWLWCX]	Mean	4.09 k[5]		9.77 d[4]	
	SD	6.56		8.09	
No. of Early Resorptions [GEN AN]	Mean	0.8 R[2]		0.7	
	SD	1.2		1.0	
	Sum	16 R[2]		14	
Early Resorptions (%) [KWLWCX]	Mean	5.04		4.62	
	SD	7.23		6.12	
No. of Late Resorptions [GEN AN]	Mean	0.1 R[2]		0.2	
	SD	0.4		0.5	

Sex: Female Day(s) Relative to Mating (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
	Sum	3 R²	(b) (4)	4	(b) (4)
Late Resorptions (%) [KWLWCX]	Mean	1.05		1.23	
	SD	2.66		3.27	
No. of Dead Foetuses [GEN AN]	Mean	0.0 R²		0.0	
	SD	0.0		0.0	
	Sum	0 R²		0	
Post-Implantation Loss [GEN AN]	Mean	0.9 R²		0.9	
	SD	1.2		1.2	
	Sum	19 R²		18	
Post-Implantation Loss (%) [KWLWCX]	Mean	6.10		5.85	
	SD	7.64		7.28	
No. of Live Foetuses [GEN AN]	Mean	13.2 R,k¹		13.1	
	SD	1.6		2.1	
	Sum	277 R,k¹		276	
No. of Male Foetuses [GEN AN]	Mean	6.1 I²		6.7	
	SD	1.7		2.0	
	Sum	129 I²		141	
No. of Female Foetuses [GEN AN]	Mean	7.0 I²		6.4	
	SD	2.1		1.5	
	Sum	148 I²		135	
Male Foetuses (%) [KWLWCX]	Mean	46.96		50.66	
	SD	14.27		10.69	
Total Litter Weight (g) [GEN AN]	Mean	64.23 ³		64.32	
	SD	5.91		10.53	
	N	21		21	
	%Diff	.		0.14	
Mean Foetal Weight (both) (g) [GEN AN]	Mean	4.89 I²		4.90	
	SD	0.23		0.30	
	N	21		21	
	%Diff	.		0.25	
Mean Foetal Weight (M) (g) [GEN AN]	Mean	5.00 I²		5.02	
	SD	0.21		0.30	
Mean Foetal Weight (F) (g) [GEN AN]	Mean	4.79 I²		4.77	
	SD	0.24		0.32	

[KWLWCX] - Kruskal Wallis & Wilcoxon
[GEN AN] - Generalised Anova/Ancova Test
1 [R,k - Automatic Transformation: Rank, (All Groups) Test: Kruskal-Wallis $p < 0.05$]
2 [I - Automatic Transformation: Identity (No Transformation)]
3 [R,kk - Automatic Transformation: Rank, (All Groups) Test: Kruskal-Wallis $p < 0.01$]
4 [d - Test: Dunnett Non-Parametric 2 Sided $p < 0.05$]

Table 70: Mean Caesarean section data

Fetal examinations
The numbers of fetuses (litters) submitted to the different examinations were as follows:

Group No.	1	2	3	4
External examination	277 (21)	(b) (4)	276 (21)	(b) (4)
Internal (visceral) examination (body)	133 (21)		132 (21)	
Fixed head examination	133 (21)		132 (21)	
Skeletal examination (head and body)	144 (21)		144 (21)	

No test article-related effects on fetal morphology were reported. This is consistent with no corresponding malformations in pups.

External observations
No test article-related effects on fetal external morphology were reported. (b) (4)

In group 3, one fetus had gastroschisis and one fetus had a small mouth and agnathia. These malformations are part of the background data for this strain of rat ((b) (4):WI(Han)) and were considered incidental in view of their isolated and sporadic nature.

Visceral observations
No test article-related effects on fetal soft tissue morphology were reported. (b) (4)

(b) (4)

One fetus of group 3 was reported with a right-sided aortic arch (b) (4) . These findings are also part of the background of findings for this strain of rat ((b) (4):WI(Han)) and were considered incidental in view of their isolated incidences.

The other less severe soft tissue anomalies and variations are part of the background data for this strain of rat and were also incidental.

[38] Kuwagata et al. Historical control data on developmental toxicity studies in rats. Congenital anomalies. 2018 59, 125-131.

Skeletal observations

No test article-related effects on fetal skeletal morphology were reported. (b) (4) . One fetus from group 3 had short and fused mandibles. These malformations associated with the abnormalities reported externally and were considered incidental in view of their isolated incidences.

As part of the background data for this strain of rat (and were considered incidental), other less severe skeletal anomalies and variations, such as supernumerary lumbar ribs, 7 lumbar vertebrae or incomplete ossification of thoracic centrum were reported.

Summary of Foetal External, Visceral and Skeletal Observations

Exam Type: Visceral Body (Rat)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		133		132	
Number of Litters Examined:		21		21	
Heart					
Heart, Ventricular septum defect - (M)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Liver					
Liver, Abnormal lobation - (A)	Fetuses N(%)	1(0.8)		0(0.0)	
	Litters N(%)	1(4.8)		0(0.0)	
Lung					
Lobe, Absent - (A)	Fetuses N(%)	0(0.0)		1(0.8)	
	Litters N(%)	0(0.0)		1(4.8)	
Lobe, Supernumerary - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Major blood vessel					
Aortic arch, Right-sided - (M)	Fetuses N(%)	0(0.0)		1(0.8)	
	Litters N(%)	0(0.0)		1(4.8)	
Ductus arteriosus, Narrowed - (M)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Subclavian artery, Malpositioned - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Umbilical artery, Transposed - (V)	Fetuses N(%)	7(5.3)		13(9.8)	
	Litters N(%)	6(28.6)		8(38.1)	

Exam Type: Skeletal Head (Rat-G21)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		144		144	
Number of Litters Examined:		21		21	
Skull					
Cranium, Acrania - (M)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Hyoid, Incomplete ossification - (A)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Interparietal, Incomplete ossification - (V)	Fetuses N(%)	3(2.1)		4(2.8)	
	Litters N(%)	3(14.3)		3(14.3)	
Mandible, Fused - (M)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Mandible, Misshapen - (A)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Mandible, Short - (M)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Parietal, Incomplete ossification - (V)	Fetuses N(%)	0(0.0)		3(2.1)	
	Litters N(%)	0(0.0) c[1]		3(14.3)	
Presphenoid, Incomplete ossification - (A)	Fetuses N(%)	1(0.7)		0(0.0)	
	Litters N(%)	1(4.8)		0(0.0)	
Squamosal, Incomplete ossification - (V)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Supraoccipital, Incomplete ossification - (V)	Fetuses N(%)	0(0.0)		2(1.4)	
	Litters N(%)	0(0.0)		2(9.5)	

1 [c - Group Factor Chi-Squared & Fisher's Exact: Test: Chi-Squared $p < 0.05$]

Exam Type: Skeletal Body (Rat-G21)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		144		144	
Number of Litters Examined:		21		21	
General					
Vertebra, Presacral vertebral arches = 27 - (A)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Forepaw					
Phalanx, Unossified - (A)	Fetuses N(%)	9(6.3)		6(4.2)	
	Litters N(%)	7(33.3)		3(14.3)	
Hindpaw					
Metatarsal, Unossified, 1st digit - (V)	Fetuses N(%)	3(2.1)		3(2.1)	
	Litters N(%)	3(14.3)		3(14.3)	
Phalanx, Unossified, proximal 2nd to 5th digits - (V)	Fetuses N(%)	46(31.9)		22(15.3)	
	Litters N(%)	11(52.4)		7(33.3)	
Ribs					
Ribs, Supernumerary cervical - (A)	Fetuses N(%)	3(2.1)		0(0.0)	
	Litters N(%)	3(14.3)		0(0.0)	
Ribs, Supernumerary lumbar - (A)	Fetuses N(%)	3(2.1)		12(8.3)	
	Litters N(%)	3(14.3)		6(28.6)	
Ribs, Thick - (A)	Fetuses N(%)	2(1.4)		4(2.8)	
	Litters N(%)	1(4.8)		3(14.3)	
Ribs, Wavy - (A)	Fetuses N(%)	0(0.0)		1(0.7)	
	Litters N(%)	0(0.0)		1(4.8)	
Ribs, Supernumerary lumbar, short - (V)	Fetuses N(%)	57(39.6)		71(49.3)	

Exam Type: Skeletal Body (Rat-G21)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		144		144	
Number of Litters Examined:		21		21	
Ribs (Continued...)					
Ribs, Supernumerary lumbar, short - (V)	Litters N(%)	17(81.0)		18(85.7)	
Sternebra					
Sternebra, Asymmetric - (A)	Fetuses N(%)	1(0.7)		0(0.0)	
	Litters N(%)	1(4.8)		0(0.0)	
Sternebra, Extra ossification site - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Sternebra, Incomplete ossification, 1st/3rd - (A)	Fetuses N(%)	1(0.7)		1(0.7)	
	Litters N(%)	1(4.8)		1(4.8)	
Sternebra, Incomplete ossification, 2nd/4th - (V)	Fetuses N(%)	1(0.7)		2(1.4)	
	Litters N(%)	1(4.8)		2(9.5)	
Sternebra, Incomplete ossification, 6th - (V)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Sternebra, Minor fusion - (A)	Fetuses N(%)	1(0.7)		0(0.0)	
	Litters N(%)	1(4.8)		0(0.0)	
Sternebra, Misshapen - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Sternebra, Unossified, 5th - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Vertebra					
Caudal, Number < 5 - (A)	Fetuses N(%)	0(0.0)		2(1.4)	

Exam Type: Skeletal Body (Rat-G21)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		144		144	
Number of Litters Examined:		21		21	
Vertebra (Continued...)					
Caudal, Number < 5 - (A)	Litters N(%)	0(0.0)		2(9.5)	
Cervical, Fused arch - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Cervical, Incomplete ossification of arch - (A)	Fetuses N(%)	0(0.0)		2(1.4)	
	Litters N(%)	0(0.0)		2(9.5)	
Cervical, Multiple abnormalities - (M)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Cervical, Odontoid process unossified - (V)	Fetuses N(%)	9(6.3)		6(4.2)	
	Litters N(%)	7(33.3)		4(19.0)	
Cervical, Unossified centrum - (V)	Fetuses N(%)	3(2.1)		2(1.4)	
	Litters N(%)	3(14.3)		2(9.5)	
Lumbar, Number = 7 - (A)	Fetuses N(%)	1(0.7)		3(2.1)	
	Litters N(%)	1(4.8)		2(9.5)	
Sacral, Misshapen arch - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Thoracic, Bipartite ossification of centrum - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Thoracic, Incomplete ossification of centrum, 1st to 9th - (A)	Fetuses N(%)	1(0.7)		3(2.1)	
	Litters N(%)	1(4.8)		3(14.3)	
Thoracic, Incomplete ossification of centrum, 10th to 13th. - (A)	Fetuses N(%)	6(4.2)		9(6.3)	

Exam Type: Skeletal Body (Rat-G21)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Fetuses Examined:		144		144	
Number of Litters Examined:		21		21	
Vertebra (Continued...)					
Thoracic, Incomplete ossification of centrum, 10th to 13th. - (A)	Litters N(%)	5(23.8) c1		9(42.9)	
Thoracic, Multiple abnormalities - (M)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	
Thoracic, Number = 14 - (A)	Fetuses N(%)	0(0.0)		0(0.0)	
	Litters N(%)	0(0.0)		0(0.0)	

Table 71: Summary of Foetal External, Visceral and Skeletal Observations

Delivery and litter data

Parturition and gestation length

No test article-related effects on parturition and gestation length were reported. In groups 1, (b) (4), 3, and (b) (4), there were 22, (b) (4), 21 and (b) (4) females that completed delivery and had liveborn pups giving a gestation index of 100%, (b) (4), 100% and (b) (4), respectively. This was consistent with the background data for this strain of rat.

In all groups, the mean duration of gestation (approximately 22 days) was comparable. (b) (4)

Pre-Birth Loss

The mean percentage pre-birth loss was higher in group (b) (4) and group (b) (4) when compared with the control group (6.8%). However, the value remained consistent with the historical control data range (from (b) (4)) for pivotal studies. Thus, the difference was considered to be incidental.

Consequently, the mean number of pups delivered was lower in groups (b) (4), respectively) compared with the control group (13.3). However, the values remained consistent with the historical control data range (from (b) (4)) for pivotal studies.

Pup Viability and Litter Sizes

(b) (4)

(b) (4)

Sex: Female Day(s) Relative to Littering (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Females Completing Delivery [CHSQFS]	N+ve	22		21	
with Liveborn Pups [CHSQFS]	N+ve	22		21	
with Stillborn Pups [CHSQFS]	N+ve	3		2	
with all Stillborn Pups [CHSQFS]	N+ve	0		0	
with all Dead PND 21 [CHSQFS]	N+ve	0		0	
Gestation Length (Days) [GEN AN]	Mean	22.1 [1]		22.0	
	SD	0.4		0.7	
	N	22		21	
Number of Implantation Sites [GEN AN]	Mean	14.3 I[3]		14.2	
	SD	2.2		2.2	
	N	22		21	
	Sum	314 I[3]		298	
Pre-Birth Loss (%) [GEN AN]	Mean	6.80 R,k[4]		8.22	
	SD	8.75		15.51	
	N	22		21	
Pups Delivered/Litter [GEN AN]	Mean	13.3 R,k[4]		13.1	
	SD	2.5		3.1	
	N	22		21	
	Sum	293 R,k[4]		276	
Live Pups PND 0 [GEN AN]	Mean	13.0 R,k[1]		13.0	
	SD	2.5		3.1	
	N	22		21	
	Sum	287 R,k[1]		274	
Live Pups PND 1 [GEN AN]	Mean	13.0 R,k[1]		13.0	
	SD	2.4		3.0	
	N	22		21	
	Sum	285 R,k[1]		273	
Live Pups Precull [GEN AN]	Mean	12.9 R,k[1]		12.9	
	SD	2.3		2.9	
	N	22		21	
	Sum	284 R,k[1]		271	
Live Pups Postcull [GEN AN]	Mean	8.0 R[3]		7.8	
	SD	0.0		1.1	
	N	22		21	
	Sum	176 R[3]		163	
Live Pups PND 7 [GEN AN]	Mean	8.0 R[3]		7.8	
	SD	0.0		1.1	
	N	22		21	
	Sum	176 R[3]		163	
Live Pups PND 10 [GEN AN]	Mean	8.0 R[1]		7.8	
	SD	0.0		1.1	
	N	22		21	
	Sum	176 R[1]		163	
Live Pups PND 14 [GEN AN]	Mean	8.0 R[1]		7.8	
	SD	0.0		1.1	
	N	22		21	

Sex: Female Day(s) Relative to Littering (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Live Pups PND 17 [GEN AN]	Sum	176 R[1]		163	
	Mean	8.0 R[1]		7.8	
	SD	0.0		1.1	
	N	22		21	
Live Pups PND 21 [GEN AN]	Sum	176 R[1]		163	
	Mean	8.0 R[1]		7.8	
	SD	0.2		1.1	
	N	22		21	
Dead, Miss., Cannib. PND 0 [CHSQFS]	Sum	175 R[1]		163	
Dead, Miss., Cannib. PND 1-4 [CHSQFS]	Sum	6		2	
Dead, Miss., Cannib. PND 5-21 [CHSQFS]	Sum	3		3	
Dead, Miss., Cannib. PND 0-21 [CHSQFS]	Sum	1		0	
Live Birth Index (%)	Mean	10		5	
Viability Index (PND 0-4) (%)		98.0		99.3	
Weaning Index (PND 4-21) (%)		99.0		98.9	
Sex Ratio PND 1 - % Males [CHSQFS]		99.4		100.0	
Sex Ratio PND 21 - % Males [CHSQFS]	Mean	51.0		48.0	
		49.7		47.6	

[CHSQFS] - Chi-Squared & Fisher's Exact
1 [R,kk - Automatic Transformation: Rank, (All Groups) Test: Kruskal-Wallis p < 0.01]
3 [I - Automatic Transformation: Identity (No Transformation)]
5 [d - Test: Dunnett Non-Parametric 2 Sided p < 0.05]

[GEN AN] - Generalised Anova/Ancova Test
2 [dd - Test: Dunnett Non-Parametric 2 Sided p < 0.01]
4 [R,k - Automatic Transformation: Rank, (All Groups) Test: Kruskal-Wallis p < 0.05]

Table 72: Delivery and litter data

Pup Clinical Observations

No test article-related effects on pup clinical observations or external abnormalities were reported.

Pup Weights
No test article-related effects on mean pup weight throughout the pre-weaning period were reported.

(b) (4)

Mean pup body weight (grams)

Sex: Female Day(s) Relative to Littering (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Mean Pup BW - Males d1 [GEN AN]	Mean	6.25 R[1]		6.27	
	SD	0.82		0.73	
	N	22		20	
	%Diff	.		0.23	
Mean Pup BW - Males d4 [GEN AN]	Mean	9.71 I[2]		9.81	
	SD	1.26		1.21	
	N	22		20	
	%Diff	.		1.00	
Mean Pup BW - Males d7 [GEN AN]	Mean	16.14 R[1]		16.47	
	SD	1.76		1.74	
	N	22		20	
	%Diff	.		2.07	
Mean Pup BW - Males d10 [GEN AN]	Mean	23.79 R[1]		24.24	
	SD	2.17		1.87	
	N	22		20	
	%Diff	.		1.87	
Mean Pup BW - Males d14 [GEN AN]	Mean	34.35 I[2]		34.93	
	SD	2.76		2.13	
	N	22		20	
	%Diff	.		1.69	
Mean Pup BW - Males d17 [GEN AN]	Mean	41.64 I[1]		42.07	
	SD	3.10		2.36	
	N	22		20	
	%Diff	.		1.04	
Mean Pup BW - Males d21 [GEN AN]	Mean	55.53 I[1]		56.10	
	SD	4.02		3.22	
	N	22		20	
	%Diff	.		1.03	
Mean Pup BW - Males d4 Postculling [GEN AN]	Mean	9.71 I[1]		9.78	
	SD	1.31		1.24	
	N	22		20	
	%Diff	.		0.66	
Mean Pup BW - Females d1 [GEN AN]	Mean	6.00 I[1]		6.06	
	SD	0.82		0.73	
	N	22		21	
	%Diff	.		0.97	
Mean Pup BW - Females d4 [GEN AN]	Mean	9.47 I[1]		9.58	
	SD	1.25		1.33	
	N	22		21	
	%Diff	.		1.25	
Mean Pup BW - Females d7 [GEN AN]	Mean	15.77 R[1]		16.10	
	SD	1.72		1.75	
	N	22		21	
	%Diff	.		2.14	
Mean Pup BW - Females d10 [GEN AN]	Mean	23.35 R[1]		23.82	
	SD	2.21		1.85	
	N	22		21	

Sex: Female Day(s) Relative to Littering (Litter: A)		Control 0mcg	BNT162b1 30mcg	BNT162b2	BNT162b3
	%Diff	.	2.73	(b) (4)	(b) (4)
Mean Pup BW - Females d14 [GEN AN]	Mean	33.71 I[2]	33.91		
	SD	2.88	1.72		
	N	22	20		
	%Diff	.	0.59		
Mean Pup BW - Females d17 [GEN AN]	Mean	40.69 I[2]	40.42		
	SD	3.16	2.18		
	N	22	20		
	%Diff	.	-0.66		
Mean Pup BW - Females d21 [GEN AN]	Mean	54.02 I[2]	53.74		
	SD	4.18	3.05		
	N	22	20		
	%Diff	.	-0.51		
Mean Pup BW - Females d4 Postculling [GEN AN]	Mean	9.49 I[1]	10.07		
	SD	1.25	1.08		
	N	22	20		
	%Diff	.	6.16		
Mean Pup Body Weight d1 [GEN AN]	Mean	6.13 R[2]	6.34		
	SD	0.82	0.49		
	N	22	20		
	%Diff	.	3.50		
Mean Pup Body Weight d4 [GEN AN]	Mean	9.60 I[1]	10.26		
	SD	1.25	1.12		
	N	22	20		
	%Diff	.	6.91		
Mean Pup Body Weight d7 [GEN AN]	Mean	15.95 R[2]	16.94 S[3]		
	SD	1.71	1.30		
	N	22	20		
	%Diff	.	6.18		
Mean Pup Body Weight d10 [GEN AN]	Mean	23.57 R[2]	24.44		
	SD	2.15	1.53		
	N	22	20		
	%Diff	.	3.66		
Mean Pup Body Weight d14 [GEN AN]	Mean	34.03 I[1]	34.50		
	SD	2.78	2.12		
	N	22	20		
	%Diff	.	1.39		
Mean Pup Body Weight d17 [GEN AN]	Mean	41.16 I[1]	41.17		
	SD	3.11	2.54		
	N	22	20		
	%Diff	.	0.02		
Mean Pup Body Weight d21 [GEN AN]	Mean	54.75 I[1]	54.71		
	SD	4.07	3.55		
	N	22	20		
	%Diff	.	-0.06		
Mean Pup BW d4 Postculling [GEN AN]	Mean	9.60 I[1]	10.32		
	SD	1.26	1.06		
	N	22	20		

Sex: Female Day(s) Relative to Littering (Litter: A)		Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
	%Diff	.	(b) (4)	1.51	(b) (4)

Table 73: Mean pup body weight (grams)

Pup Physical and Functional Development

No test article-related effects on pre-weaning physical (pinna unfolding and eye opening) and functional (pupil and auditory reflexes) development were reported.

Summary of reflex and physical development

Group	1	2	3	4
	Control	BNT162b1	BNT162b2	BNT162b3
Dose level	0 µg	(b) (4)	30µg	(b) (4)
PINNA UNFOLDING - % of pups positive:				
day 1 *post-partum*	5		6	
day 2 *post-partum*	51		51	
day 3 *post-partum*	98		99	
day 4 *post-partum*	100		100 (3)	
EYE OPENING - % of pups positive:				
day 12 *post-partum*	0		3	
day 13 *post-partum*	19		9	
day 14 *post-partum*	83		79	
day 15 *post-partum*	99		96	
day 16 *post-partum*	100		100	
day 17 *post-partum*				
PUPILLARY REFLEX - day 21 *post-partum* - % of pups positive:	100		100	
AUDITORY REFLEX - day 21 *post-partum* - % of pups positive:	100		100	

(1): 99.6%
(2): values excluded for three pups that were not observed after PND14 in error
(3): 99.7%, one unselected pup for culling was not observed after PND4
*: $p \leq 0.05$; *** $p \leq 0.001$

Table 74: Summary of reflex and physical development

Pup Necropsy Findings

No test article-related effects on pup macroscopic observations or malformations were reported.

Necropsy Findings of Adult Females

Test article-related macroscopic findings were reported at the injection sites (firm area, enlarged, edematous area and/or pale). These findings were consistent with the administration of the vaccine and an inflammatory/immune response localized to the injection site.

Across all groups (including controls), abnormalities of the liver (diaphragmatic hernia, mottled surface, abnormal shape or adherent mass) were reported for isolated females and were considered incidental.
Across all groups (including controls), alopecia and/or sores/crusts were also reported for isolated females and were considered incidental.

Summary of maternal macroscopic observations

Removal Reason: TERMINAL SACRIFICE	FEMALES			
	Control 0mcg	BNT162b1 (b) (4)	BNT162b2 30mcg	BNT162b3 (b) (4)
Number of Animals on Study :	44		43	
Number of Animals Completed:	(44)		(43)	
LIVER;				
Submitted	(2)		(1)	
No Visible Lesions	0		0	
Hernia; diaphragm; between right and left median lobes	2		0	
Mottled surface; all lobes	0		0	
Abnormal shape; left median lobe	0		0	
Small; left median lobe	0		0	
Mass a; adherent to surrounding tissue; papillary process; solid; dark; heterogeneous	0		1	
IDENTIFICATION;				
Submitted	(3)		(12)	
No Visible Lesions	3		12	
SKIN/SUBCUTIS;				
Submitted	(2)		(6)	
No Visible Lesions	0		0	
Alopecia; single; forelimb; right; left	0		3	
Alopecia; single; forelimb; left	1		0	
Alopecia; single; abdominal region; thoracic region	0		0	
Alopecia; single; thoracic region	0		1	
Alopecia; single; thoracic region; abdominal	0		1	
Alopecia; right; forepaw; abdominal; left	0		0	
Sore/crust; many; back; head	0		1	
Sore/crust; many; forelimb; left	0		0	
Sore/crust; single; right	0		0	
Sore/crust; single; forelimb; right	0		1	
Sore/crust; single; hindlimb; left	1		0	
Sore/crust; single; abdominal region	2		0	
NO CORRELATE;				
Submitted	(9)		(5)	
NO CORRELATE; (continued)				
No Visible Lesions	0		0	
No correlate	9		6	
INJECTION SITE 1;				
Submitted	(0)		(9)	
No Visible Lesions	0		9	
Pale	0		0	
INJECTION SITE 2;				
Submitted	(0)		(10)	
No Visible Lesions	0		0	
Firm area	0		9	
Enlarged	0		8	
Oedematous area	0		1	
Pale	0		4	
NO CORRELATE;				
Submitted	(0)		(0)	
No Visible Lesions	0		0	
No correlate	0		0	
LIVER;				
Submitted	(0)		(0)	
No Visible Lesions	0		0	
Pale; all lobes	0		0	
SPLEEN;				
Submitted	(0)		(0)	
No Visible Lesions	0		0	
Enlarged	0		0	
IDENTIFICATION;				
Submitted	(0)		(0)	
No Visible Lesions	0		0	
SKIN/SUBCUTIS;				
Submitted	(0)		(0)	
No Visible Lesions	0		0	
Alopecia; single; forelimb; abdominal region; left	0		0	

Table 75: Summary of maternal macroscopic observations

Summary

(b) (4), BNT162b2 (b) (4)) resulted in clinical signs and macroscopic findings localized to the injection site as well as transient body weight and food consumption effects after each dose administration. These maternal findings might be related to the administration of the vaccine and an inflammatory/immune response.

No test article-related effects on estrous cycles, pre-coital interval, mating, fertility and pregnancy index, or on any ovarian, uterine, or litter parameters, including F1 survival, growth, external, visceral, and skeletal morphology, or effects on pre-weaning physical and functional development of the F1 pups were reported.

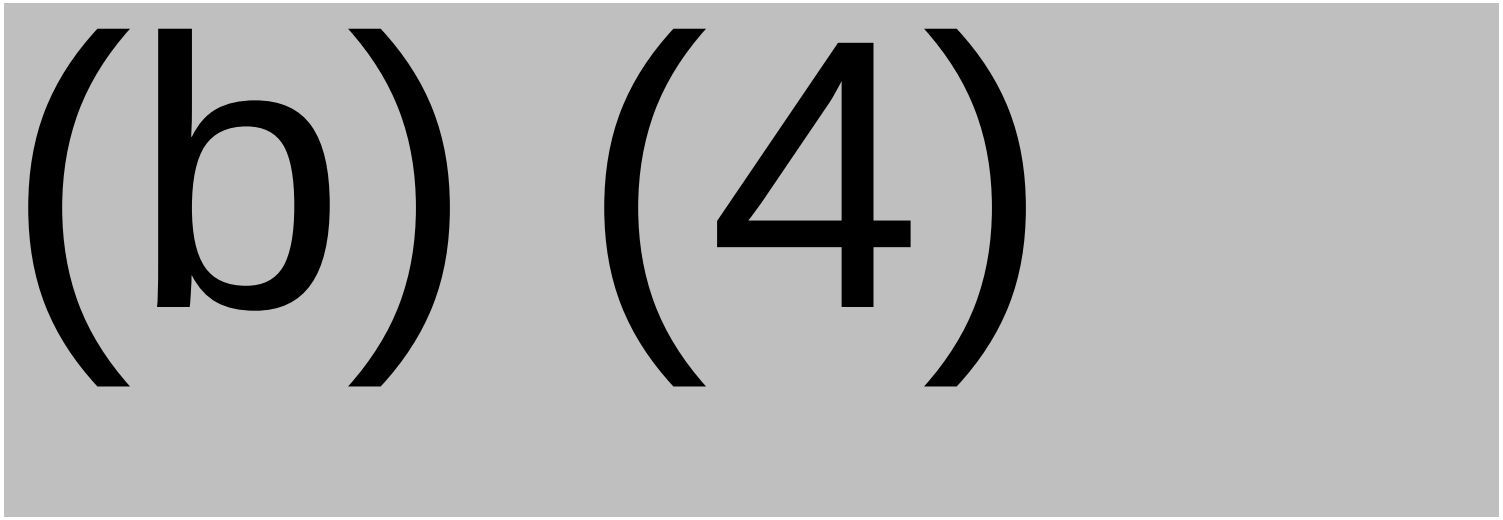
Four doses (2 prior to mating and 2 during gestation) administration of the test articles ((b) (4), BNT162b2, (b) (4)) elicited SARS-CoV-2 neutralizing antibody responses in the majority of females just prior to mating (M-14), at the end of gestation (GD21), and at the end of lactation (LD21). Also, SARS-CoV-2 neutralizing titers were detected in most offspring (fetuses on GD21 and pups on PND21). Prior to vaccine administration or in saline-administered control animals, SARS-CoV-2 neutralizing antibody titers were not reported.

Conclusion

Test article-related effects on body weight, food consumption, and effects localized to the injection site after each dose administration were reported. No test article-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 were reported.

Test article-related immune responses were confirmed in F0 female rats following administration of each vaccine candidate and these responses were also detectable in the F1 offspring (fetuses and pups).

6 pages have been determined to be not releasable: (b)(4)



For complete historical data, please visit appendix 29 on page 1084 of the study report submitted in amendment number 165.

References:

1. Habibzadeh P, Stoneman EK. The Novel Coronavirus: A Bird's Eye View. Int J Occup Environ Med. 2020; 11 (2): 65-71.
2. Vogel AB, Lambert L, Kinnear E, Busse D, Erbar S, Reuter KC, et al. Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses. Mol Ther. 2018; 26 (2): 446-55.
3. Moyo N, Vogel AB, Buus S, Erbar S, Wee EG, Sahin U, et al. Efficient Induction of T Cells against Conserved HIV-1 Regions by Mosaic Vaccines Delivered as Self-Amplifying mRNA. Mol Ther Methods Clin Dev. 2018; 12: 32-46.
4. Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature. 2017; 543 (7644): 248-51.
5. Colombo S, Buclin T, Décosterd LA, Telenti A, Furrer H, Lee BL, Biollaz J, Eap CB (October 2006). "Orosomucoid (alpha1-acid glycoprotein) plasma concentration and genetic variants: effects on human immunodeficiency virus protease inhibitor clearance and cellular accumulation". Clinical Pharmacology and Therapeutics. 80 (4): 307–18. doi:10.1016/j.clpt.2006.06.006. PMID 17015049.
6. Urien S, Brée F, Testa B, Tillement JP (November 1991). "pH-dependency of basic ligand binding to alpha 1-acid glycoprotein (orosomucoid)". The Biochemical Journal. 280 (Pt 1) (1): 277–80. PMC 1130632. PMID 1741754.
7. Zimmermann-Belsing T, Rasmussen AK, Feldt-Rasmussen U, Bøg-Hansen TC (February 2002). "The influence of alpha1-acid glycoprotein (orosomucoid) and its glycoforms on the function of human thyrocytes and CHO cells transfected with the human TSH receptor". Molecular and Cellular Endocrinology. 188 (1–2): 241–51. doi:10.1016/s0303-7207(01)00650-5. PMID 11911961.

8. Dietrich JW, Landgrafe G, Fotiadou EH (2012). "TSH and thyrotrophic agonists: key factors in thyroid homeostasis". Journal of Thyroid Research. 2012: 351864. doi:10.1155/2012/351864. PMC 3544290. PMID 23365787.
9. Fischer K, Kettunen J, Würtz P, Haller T, Havulinna AS, Kangas AJ, Soininen P, Esko T, Tammesoo ML, Mägi R, Smit S, Palotie A, Ripatti S, Salomaa V, Ala-Korpela M, Perola M, Metspalu A (February 2014). "Biomarker profiling by nuclear magnetic resonance spectroscopy for the prediction of all-cause mortality: an observational study of 17,345 persons". PLoS Medicine. 11 (2): e1001606. doi:10.1371/journal.pmed.1001606. PMC 3934819. PMID 24586121.
10. Ulich TR, del Castillo J, Souza L. Kinetics and mechanisms of recombinant human granulocyte-colony stimulating factor-induced neutrophilia. Am J Pathol 1988;133(3):630-38.
11. Abreu R, Quinn F, Giri PK. Role of the hepcidin-ferroportin axis in pathogen-mediated intracellular iron sequestration in human phagocytic cells. Blood Adv 2018;2(10): 1089-100.
12. Brooks MB, Turk JR, Guerrero A, et al. Non-Lethal Endotoxin Injection: A Rat Model of Hypercoagulability. PLoS One 2017;2(1),e0169976.
13. Kim A, Fung E, Parikh SG, et al. A mouse model of anemia of inflammation: complex pathogenesis with partial dependence on hepcidin. Blood 2014;123(8):1129-136.
14. Wrighting DM and Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood 2006;108(9):3204-09.
15. Ivens IA, Achanzar W, Baumann A, et al. PEGylated biopharmaceuticals: current experience and considerations for nonclinical development. Toxicol Pathol 2015 Oct;43(7):959-83.