[redacted] Report No. 38166
Amendment No. 1 to Final Report

[redacted]

REPEAT-DOSE TOXICITY STUDY OF THREE LNP-FORMULATED RNA PLATFORMS ENCODING FOR VIRAL PROTEINS BY REPEATED INTRAMUSCULAR ADMINISTRATION TO WISTAR HAN RATS

(Final Report dated 01 July 2020)

Sponsor:
[redacted]

Study conducted by:
[redacted]

Contact person:
[redacted]

Contact person:
[redacted]

17 September 2020

This Amendment No. 1 to Final Report consists of 2233 pages and 4 pages.
This is page I.

REASONS FOR AMENDMENT NO. 1 TO FINAL REPORT

Upon request of the Sponsor, minor layout changes and/or corrections are made to the report that do not affect the validity and scientific results or the conclusions of the final report.

The following changes were made:

1. SUMMARY - FINDINGS

To improve readability and provide a more comprehensive summary, all Text tables are deleted from Section 1.2 and replaced with references to the respective Text tables in the results section of the report.

Furthermore, in Section 1.2 in the paragraph on local tolerance the finding of eschar formation was incorrectly described with occurrence on test days 14 and 15 instead of on test day 14 only. Additionally, in the paragraph on haematology and coagulation the finding of an increased number of eosinophils in groups [redacted] and 7 was missing. Further, in the paragraph on clinical chemistry the directions of changes for albumin and globulin levels were incorrectly stated as an increase in albumin and a decrease in globulin plasma levels instead of a decrease in albumin and an increase in globulin plasma levels.

In this Amendment No. 1 to Final Report the incorrect finding of eschar formation and the incorrect directions of changes for albumin and globulin are corrected, and the finding of an increased number of eosinophils in groups [redacted] and 7 is added.

2. TEST ITEM

In Section 2.3.5 on test item no. 5, the designation for the test item was incorrectly given as 'modRNA' instead of 'saRNA'.

In this Amendment No. 1 to Final Report the incorrect designation is corrected.

3. RESULTS

In Section 4.7 the finding of an increased number of eosinophils in groups [redacted] and 7 was missing. Additionally, in Section 4.8 the directions of change for albumin and globulin levels were incorrectly stated as an increase in albumin and a decrease in globulin plasma levels instead of a decrease in albumin and an increase in globulin plasma levels.

In this Amendment No. 1 to Final Report the incorrect directions of changes for albumin and globulin are corrected, and the finding of an increased number of eosinophils in groups [redacted] and 7 is added.

4. TABLES

In Table 1 2 (Local Tolerance Erythema, Oedema, Induration, Hardening) the incorrect test item [redacted] instead of [redacted] was stated for [redacted] In addition, the grading scale as given in the footnote was specific for erythema only, and the gradings for oedema and indurations were missing.

In this Amendment No. 1 to Final Report the incorrect test item is corrected. The grading scale given in the footnote is referred to erythema, and additionally, for oedema and indurations the respective grading scales are given as a reference to Section 3.8.3.

5. ADDITIONAL CHANGES

In Section 4. 1, the main sentence of the last sentence of the second paragraph was missing the verb "was noted", which is now added to the text (i.e. "[...] was noted on test day 14 [...]").

The following typing errors in the Final Report are corrected by this Amendment No. 1 to Final Report:

Quality Assurance Statement: Year of the Study Plan '16 March 2020' instead of '16 March 2019'.

Section 4. 7: In Text table 4 9, for the parameter "eosinophils" the sex is corrected from 'm' to 'f'.

Section 4.8: In Text table 4 12, for the parameter "globulin" the entry 'Group: [redacted] Sex: m, Test day: 17, Change: + 9.5% * *' is corrected to 'Group: [redacted] Sex: f, Test day: 4, Change: + 9.5% *'.

This amendment does not affect the validity of the data.

[redacted]
Study Director

17 Sep 2020
Date

This Amendment No. 1 to Final Report has been audited by the Quality Assurance Unit (QAU) and is considered to be an accurate account of the project.

[redacted]

17. Sep. 2020
Date

[redacted] Report No. 38166

[redacted]

REPEAT-DOSE TOXICITY STUDY OF THREE LNP-FORMULATED RNA PLATFORMS ENCODING FOR VIRAL PROTEINS BY REPEATED INTRAMUSCULAR ADMINISTRATION TO WISTAR HAN RATS

Sponsor:
[redacted]

Study conducted by:
[redacted]

Contact person:
[redacted]

Contact person:
[redacted]

17 September 2020

[redacted]

LIST OF CONTENT

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STATEMENT OF COMPLIANCE

REPEAT-DOSE TOXICITY STUDY OF THREE LNP-FORMULATED RNA PLATFORMS ENCODING FOR VIRAL PROTEINS BY REPEATED INTRAMUSCULAR ADMINISTRATION TO WISTAR HAN RATS

The study was performed in compliance with:

- 'Good Laboratory Practice' Regulations of the EC enacted in Germany in the 'Chemikaliengesetz' [Chemicals Act], current edition;
- 'OECD Principles of Good Laboratory Practice' Document No. 1 (ENV/MC/CHEM (98) 17) regulated in the Directive 2004/10/EC of the European Parliament and the Council of 11 February 2004.

These principles are compatible with 'Good Laboratory Practice' (GLP) regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA) and Japan (MHLW, MAFF, and METI). Animal husbandry is performed in compliance with EU Welfare Standards (Directive 2010/63/EU).

Raw data obtained during the performance of the study are accurately reflected.

The analysis of dose exposure was conducted under the responsibility of the Sponsor and is excluded from this statement.

[redacted]
Study Director

17 Sep 2020
Date

QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the raw data for the study. Methods, procedures and observations are correctly and completely described in the report:

REPEAT-DOSE TOXICITY STUDY OF THREE LNP-FORMULATED RNA PLATFORMS ENCODING FOR VIRAL PROTEINS BY REPEATED INTRAMUSCULAR ADMINISTRATION TO WISTAR HAN RATS

Study Plan dated 16 March 2020 and 9 Study Plan amendments.

Text table 1: Inspections of [redacted] QAU

Date of inspection	Criteria	Date of report to the Study Director and the Management
16 Mar 2020	Study Plan.	16 Mar 2020
17 Mar 2020	Sponsor's visit: Prearrangements for test item preparation, administration, body temperature, local tolerance, documentation.	17 Mar 2020
24 Mar 2020	Body weight, blood withdrawal and processing for cytokine determination, time points of blood withdrawal, labels, administration, body temperature, local tolerance, documentation.	24 Mar 2020
31 Mar 2020	Blood withdrawal and processing for cytokine determination, time points of blood withdrawal, labels, administration, body temperature, local tolerance, documentation.	31 Mar 2020
08 Apr 2020	Urine collection, blood withdrawal and processing for laboratory examinations and for dose exposure analysis, dissection, organ removal, organ weights, documentation.	08 Apr 2020
21 Apr 2020	Body temperature, administration, blood withdrawal and processing, kinetics, labels, local tolerance, documentation.	21 Apr 2020
23 Apr 2020	Blood withdrawal and processing for laboratory examinations, dose exposure analysis and cytokine analysis, animal sacrifice, dissection, organ removal, organ weights, bone marrow smears, documentation.	23 Apr 2020
26 June and 29 June to 01 July 2020	Final Report.	01 July 2020
15 and 17 Sep 2020	Amendment to Final Report.	17 Sep 2020

In addition to the detailed study-based inspections, series of routine facility inspections were also conducted and reported to the Management.

Approved and submitted by: [redacted]

17. Sep. 2020
Date

EXPLANATIONS AND ABBREVIATIONS

Symbols

[1] - [n]	reference to footnotes in text
#(n)	reference to footnotes in text tables
( )	animal number in the tables section
%	per cent
!	refer to result comment at the end of the table
...n	inappropriate for statistics (number of individual data values is less than 3 or all values were below lowest level of quantification, e.g. for cytokines)
↑	increase relative to study control range, but % difference not quantifiable due to lacking concurrent controls
↓	decrease relative to study control range, but % difference not quantifiable due to lacking concurrent controls

Letters and acronyms

% Diff	percent difference (from control group)
a.m.	ante meridiem
abs.	absolute
ANOVA	analysis of variance
approx.	approximately
b.w.	body weight
EC	European Commission
EPA	Environmental Protection Agency (USA)
EU	European Union
f	female
FDA	Food and Drug Administration (USA)
GLP	Good Laboratory Practice
i.e.	id est (that is)
i.m.	intramuscular
Inj.	injection
LLOQ	lower limit of quantification
LNP	lipid nanoparticles
[redacted]	[redacted]
LUFA-ITL	Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Institut für Tiergesundheit und Lebensmittelqualität
m	male
MAFF	Ministry of Agriculture, Forestry and Fisheries (Japan)
METI	Ministry of Economy, Trade and Industry (Japan)
MHLW	Ministry of Health, Labour and Welfare (Japan)
MS	main study
n, N	number (e.g. group size, sample size)
n/a	not applicable
ns	not statistically significant
OECD	Organization for Economic Co-Operation and Development
p.a.	post administration
p.m.	post meridiem
PBS	phosphate-buffered saline
PEG	polyethylene glycol
PrDs	predose

- Letters and acronyms continued on the next page -

Letters and acronyms *(continued)*

QAU	Quality Assurance Unit
RBD	receptor-binding domain
rel.	relative
RNA	ribonucleic acid
mRNA	messenger RNA
uRNA	uridine mRNA
modRNA	nucleoside-modified mRNA
saRNA	self-amplifying mRNA
RP	recovery period
SA	satellite animals
SARS	severe acute respiratory syndrome
SARS-CoV	SARS coronavirus
SD	standard deviation
SOP	Standard Operating Procedure
TD	test day
TS	terminal sacrifice
TW	test week
USA	United States of America

Weights and measures

°C	degree Celsius
cm	centimetre
dL	decilitre
fL	femtolitre
fmol	femtomole
g	gram
h	hour
kg	kilogram
L	litre
mg	milligram
min	minute
µg	microgram
µL	microlitre
µmol	micromole
mL	millilitre
mm	millimetre
mmol	millimole
sec	second
U	unit

Measuring units

Body weight in g
Food intake in g/kg b.w./day
Absolute organ weights in g
Relative organ weights in g/kg b.w.
Relative urine volume in mL/kg b.w./24 h

Haematology / Coagulation

aPTT	activated partial thromboplastin time
Baso	basophilic granulocytes

- Haematology / Coagulation continued on the next page -

Haematology / Coagulation *(continued)*

Eos	eosinophilic granulocytes
HCT	haematocrit
HGB	haemoglobin
LUC	large unclassified cells
Lym	lymphocytes
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
Mono	monocytes
MPC	mean platelet component
MPV	mean platelet (thrombocyte) volume
Neut	neutrophilic granulocytes
PDW	platelet distribution width
PLT	platelets
PT	prothrombin time
RBC	red blood cell count (= erythrocytes)
RDW	red cell distribution width
Reti	reticulocytes
WBC	white blood cell count (= leucocytes)

Clinical chemistry

Alb.	albumin
ALAT	alanine aminotransferase
aP	alkaline phosphatase
ASAT	aspartate aminotransferase
CK	creatine kinase
Glob.	globulin
Gamma-GT	gamma-glutamyltransferase
LDH	lactate dehydrogenase

Cytokines

IFN-gamma	interferon-alpha
IL-1beta	interleukin-1 beta
IL-6	interleukin-6
IL-10	interleukin-10
TNF-alpha	tumour necrosis factor-alpha

Urinalysis

+	'small amount' of analyte/few in some fields examined
+ +	'moderate amount' of analyte/few in all fields examined
+ + +	'large amount' of analyte/many in all fields examined
ery	erythrocyte
LC	lemon-coloured
neg	negative/none found in any field examined
pos	positive
SC	straw-coloured

Histopathology

For explanations see the 'Histopathological Report' in Section 6.

Test item nomenclature

For reasons of better readability and due to space restrictions, the dose levels used in the study are referred to using the designations given below instead of the full designations as given in Section 2.3.

[redacted]

Group 7: **100 µg BNT162b2/animal**

1. SUMMARY

1.1 Conduct of study

Test items	[redacted] 3) BNT162b [redacted]
Control item	Buffer (PBS/300 mM Sucrose)
Test item batch nos.	1) CoVVAC/090320 2) CoVVAC/100320 3) CoVVAC/160320 4) CoVVAC/130320
Control item batch no.	090320
Test species / Strain / Stock	Rat / Wistar / Crl:WI(Han)
Breeder	[redacted]
Number and sex of animals	255 animals (126 + 3[1] males and 126 females)
Route of administration	Intramuscular (i.m.) administration into the Musculus biceps femoris using a Microfine+ Syringe 0.5 mL, 0.33 mm (29G) × 12.7 mm (BD, 324824).
Frequency of administration	Groups 1 to [redacted] and 7: On test days 1, 8 and 15; in total 3 administration days at one-week intervals per animal. [redacted]
Administration volume	Groups 1, [redacted] and 7: 100 µL/administration site; 2 administration sites In total 200 µL/animal/administration day [redacted]

[1] Due to a short-term change of test item and dose level for group 3, three animals had already been treated with the originally planned test item and dose. These three animals were replaced by 3 spare animals for the correct teatment.

[redacted]

Dosages

Groups 1 to 7:
Group 1: Control (200 µL Buffer/animal)
[redacted]
Group 7: 100 µg BNT162b2/animal
[redacted]

Duration of study

- 5 to 11 adaptation days
- 17 test days for groups 1 to [redacted] and group 7 [redacted]
- 3 additional weeks for the animals scheduled for the recovery period

1.2 Findings

Local tolerance

Treatment period

Local reactions were observed in male and female animals treated intramuscularly with [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15, [redacted] The incidence and severity of the reactions were higher after the 2nd or 3rd injections compared with the 1st injection. The majority of animals revealed very slight to moderate oedema at the injection site(s) following the 1st, 2nd, and/or 3rd injection of the respective test item. A few animals had severe oedema at [redacted] 24 h after the 3rd injection of **100 µg BNT162b2/animal**. The majority of these observations of oedema were resolved or showed signs of resolution by 144 h postdose.

For a few animals, slight or well-defined erythema was also observed in test-item administered animals after the 1st, 2nd, and/or 3rd injection. In addition, after the 2nd or 3rd injection, transient observations of severe erythema were seen in all test article-dosed groups, [redacted] starting at 96 h after administration. [redacted]

An indurated and/or thickened injection site, partly accompanied by incrustation, was noted for nearly all animals in all treatment groups at macroscopic inspection at necropsy.

The microscopic examination revealed that test item-related injection site reactions were present in all groups and characterized by mostly moderate inflammation (up to marked) in males

and moderate inflammation in females. The most severe findings were consistently in animals administered [redacted] **100 µg BNT162b2/animal**, [redacted] The inflammation was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis, at the injection site. Injection site inflammation was associated with mostly moderate oedema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. [redacted]. 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).

Microscopic injection site findings correlated with macroscopic observations of thickening, induration, and incrustation. Injection site findings were consistent with an immune/inflammatory response to intramuscular vaccine administration.

Recovery period

The local skin reactions and the indurations and/or thickenings noted macroscopically for the muscle at the injection site(s) were resolved at the end of the recovery period.

Most of the microscopic findings noted 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, and skeletal muscle) partially or fully recovered at the end of the 3-week recovery period. Some inflammatory lesions were still noted at the injection sites and the surrounding tissue of some animals.

Clinical signs

Treatment and recovery period

None of the male and female animals treated intramuscularly with [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15 (3 administrations), [redacted] revealed any test item-related systemic changes in

behaviour, external appearance, or consistency of faeces.

Mortality

[redacted] **BNT162b2,** [redacted]

Treatment and recovery period

No test item-related deaths were noted for any treatment.

Body weight and body weight gain

[redacted] **BNT162b2** [redacted]

Treatment period

Slightly decreased body weights and body weight gain were present in all test-item treated groups compared to controls. Body weight decreases were primarily due to decreases in body weight 24 h after dosing. However, body weight gain during the inter-dosing interval was similar to controls.

In summary, the absolute body weight was affected, but the body weight gain between dosing was not.

Recovery period

No noteworthy changes were noted.

Food and drinking water consumption

Treatment period

[redacted]

[redacted] **BNT162b2,** [redacted]

No test item-related influence was observed on the food intake and the drinking water consumption.

Recovery period

No noteworthy changes were noted.

Body temperature

Treatment period

[redacted] **BNT162b2,** [redacted]

Intramuscular administration with [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15, [redacted] led to slightly increased body

temperatures at 4 h p.a. and/or 24 h p.a. compared to the control animals. The effect appeared to be slightly more pronounced in the groups treated with the higher test item dose levels (i.e. groups [redacted] and 7).

Recovery period

During the recovery period, the body temperature remained at a slightly higher level compared to the control group in all previously test item treated groups.

Haematology and coagulation

Treatment period

The most consistent test item-related haematological changes were dose-related increases in neutrophils and large unstained cells (LUC), which were seen with all test items on test day 17, but were greatest in groups [redacted] and 7 and were greater in females relative to males. Other test item-related changes included decreases in the absolute and relative reticulocyte count (test day 4 only), platelet count, and red cell mass (HGB, HCT and RBC; test day 17 only), and increases in the numbers of leucocytes, monocytes, eosinophils, basophils and/or fibrinogen concentrations.

[redacted]

[redacted]

[redacted]

BNT162b2 - Group 7
Test item-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, eosinophils, large unstained cells (LUC), basophils and/or the levels of fibrinogen as given in Text table 4-8.

Recovery period
All changes fully reversed by the end of the recovery phase.

Clinical chemistry

Treatment period
[redacted] **BNT162b2,** [redacted]
An elevated plasma activity of gamma-glutamyltransferase (gamma-GT) was noted for all test item-treated groups in comparison to the control group as given in Text table 4-10. There were no macroscopic or microscopic findings consistent with cholestasis or hepatobiliary injury to explain the increased gamma-GT activity.

Further, a decrease in albumin plasma levels and an increase in globulin plasma levels, resulting in an altered albumin/globulin ratio, were observed in all test item treated groups. The 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.

Recovery period
The elevated plasma activity of gamma-GT had subsided in all previously test item-treated groups.

Urinalysis

Treatment and recovery period
No test item-related changes were noted.

Immunogenicity assessment (performed by [redacted])	The available data demonstrates that all BNT162 vaccine candidates elicited a SARSCoV-2 spike protein specific antibody response directed against the S1 domain and the RBD sub-domain. Antibody responses detected via ELISA directly translated into neutralizing activity as seen in the VSV/SARS-CoV2-S pseudovirus neutralization test with BNT162 vaccines showing higher antigen-specific antibody titers also displaying more pronounced virus neutralization effect. A comparison of the three RNA platforms with regard to their immunogenicity in rats may not be fully predictive for how they may perform relative to each other in human due to species-specific differences in immunity mechanisms.
Ophthalmological and auditory examination	No changes were noted.
Acute phase proteins	Treatment period [redacted] **BNT162b2,** [redacted] Elevated serum levels of the acute phase proteins alpha1-acid glycoprotein and alpha2 macroglobulin were noted for all test item-treated groups in comparison to the control group on test day 4 and test day 10 to 17 as given in Text table 4-14 and Text table 4-15. Recovery period The elevated serum levels of alpha1-acid glycoprotein and alpha2 macroglobulin noted during the treatment period had subsided in all previously test item-treated groups.
Cytokines	Treatment and recovery period [redacted] **BNT162b2,** [redacted] No test item-related changes were noted for any treatment. There were no general differences between the test item-treated groups and the control group and among the various test item-treated groups.
Macroscopic *post mortem* findings	Terminal sacrifice [redacted] **BNT162b2,** [redacted] Test item-related findings were noted for all test items and all dose levels in male and female animals as given in Text table 4-19, Text table 4-20 and Text table 4-21. All changes noted macroscopically were interpreted to be due to inflammation at the injection site and/or immune activation.

Recovery sacrifice
All macroscopic findings noted in the spleen had subsided at the end of the 3-week recovery period.

Enlarged iliac lymph nodes were still noted for a few animals at the end of the 3-week recovery

[redacted]

Group 7
(100 μg BNT162b2/animal): One of 5 males, 3 of 5 females.

Organ weights

Terminal sacrifice
[redacted] **BNT162b2,** [redacted]
The macroscopic findings of enlarged spleens correlated with increased relative and absolute spleen weights and are identified in Text table 4-22.

Recovery sacrifice
There were no noteworthy differences in the organ weights between the previously test item-treated animals and the control animals after 3-weeks of recovery.

Histopathology

Terminal sacrifice
Test item-related microscopic findings at the end of dosing were evident in injection sites and surrounding tissues, increased cellularity of germinal centres and increased plasma cells in the draining (iliac) lymph nodes, bone marrow, spleen, and liver.

For details on the findings at the injection sites refer to 'Local tolerance' further above.

Test item-related findings in the draining (iliac) lymph node were characterized by increased cellularity of the follicular germinal centres and increased plasma cells (plasmacytosis) which were variably present in all groups.

Test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary haematopoiesis in the spleen (which correlated with increased spleen size and weight), and a test item-related vacuolation of hepatocytes in the portal regions of the liver were present in all groups. The liver findings were not associated with changes in markers of hepatocyte injury

(e.g. ALAT). While gamma-GT was elevated in test-item treated animals, it is not a marker of hepatocyte injury.

The test item-related findings are summarised in Text table 4-24, Text table 4-25 and Text table 4-26.

Recovery sacrifice

Most of the microscopic findings noted 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 at the end of the recovery period. Some inflammatory lesions were still noted at the injection sites and the surrounding tissue of some animals, being less severe (minimal to mild) if not resolved; plasmacytosis in the iliac lymph node was less severe and present in fewer groups [redacted] or **100 µg BNT162b2/animal**), indicating partial or complete recovery.

The test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary haematopoiesis in the spleen, and the vacuolation of hepatocytes in the portal regions of the liver were fully recovered.

1.3 Conclusion

Intramuscular administration of 4 LNP-formulated RNA vaccines (based on 3 LNP-formulated RNA platforms) encoding viral proteins once weekly for 2 or 3 administrations to male and female Wistar Han rats was tolerated without evidence of systemic toxicity and produced the expected local inflammatory reaction. Treatment groups included: [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15 (3 administrations), [redacted]

No test item-related deaths were noted for any treatment. There were no test-item related ophthalmologic or auditory alterations. None of the animals of any treatment group revealed any test item-related systemic changes in behaviour, external appearance, or consistency of faeces.

Clinical findings included slightly decreased body weights and body weight gain and transient slight elevations in body temperatures at 4 and 24 h after dosing for all test-item treated groups compared to controls. Body weight decreases were primarily due to decreases in body weight 24 h after dosing; however, body weight gain during the inter-dosing interval was similar to controls. [redacted]

Test-article related injection site observations included oedema, erythema, and induration; oedema was the most common finding, followed by erythema, and very rarely induration. The incidence was higher and observations were more severe after the second and or third dose administration compared to the first administration, but resolved prior to subsequent dosing and were fully recovered at the end of the 3-week recovery period. Macroscopic findings at the injection sites included induration or thickening, occasionally accompanied by incrustation, which was noted for nearly all test article-treated animals. This correlated microscopically to inflammation in all test article-administered animals. Inflammation was mixed to mononuclear with variable fibrosis, oedema, and myofiber degeneration (rare necrosis).

Inflammation was most severe in animals dosed with [redacted] **100 µg BNT162b2/animal**, [redacted] The findings were typical of an inflammatory response to vaccine antigen and lipid nanoparticle. Inflammation was occasionally evident extending into tissues adjacent to the injection site. Inflammation at the injection site was accompanied by elevations in circulating white blood cells (granulocytes, monocytes, and LUC) and acute phase proteins (fibrinogen, alpha-2 macroglobulin, and alpha-1 acid glycoprotein).

At the end of the 3-week recovery phase, all clinical injection site findings, clinical pathology findings and macroscopic observations had resolved and there was evidence of recovery of the injection site inflammation microscopically. The injection site findings were not interpreted as adverse because of limited severity, lack of systemic findings, and absence of clinical signs of lameness.

Test-article related macroscopic enlargement of the draining (iliac) lymph nodes was evident at the end of dosing. Microscopically, this finding correlated with increased cellularity of germinal centres and increased plasma cells in the draining (iliac) lymph node and is an anticipated immune response to the administered vaccine and LNP. At the end of the 3-week recovery, a few animals treated with [redacted] **BNT162b2**[redacted] and/or 100 μg/animal) still had slightly enlarged iliac lymph nodes. All other test item-related changes had subsided.

Effects considered secondary to immune activation/acute phase responses and inflammation at the injection site included transient reticulocyte decreases (test day 4 only), minimal decreases in RBC, HGB, and HCT on test day 17 only, and sporadic small magnitude decreases in platelets. Platelet reductions were likely due to inflammation-related platelet activation and consumption and were un-associated with other alterations in haemostasis. These effects had subsided after the 3-week recovery period.

Test-article related macroscopic enlargement of spleen and associated absolute and relative spleen weights correlated microscopically to increased haematopoiesis; this finding was resolved at the end of the 3-week recovery period. Increased haematopoiesis was also evident in the bone marrow. Both findings were secondary to inflammation at the injection site and were fully resolved at the end of the 3-week recovery period.

Test-article related microscopic vacuolation of portal hepatocytes was present in all groups, with a higher incidence in females than males for all but the groups that were administered **100 µg/animal** [redacted] or **BNT162b2**. This finding was not adverse because it was unassociated with alterations in hepatic function (e.g. no elevations in ALAT) and was fully reversed at the end of the 3-week recovery period. This change may be related to hepatic clearance of the pegylated lipid in the LNP.

No test item-related changes were observed for cytokine serum levels.

Elevations in GGT were evident in all test-item treated animals. There were no macroscopic or microscopic findings consistent with cholestasis or hepatobiliary injury to explain the increased gamma-GT activity which was completely resolved at the end of the 3-week recovery period.

Immunogenicity assessment demonstrated that all BNT162 vaccine candidates elicited a SARSCoV-2 spike protein specific antibody response directed against the S1 domain and the RBD sub-domain. Antibody responses detected via ELISA directly translated into neutralizing activity as seen in the VSV/SARS-CoV2-S pseudovirus neutralization test with BNT162 vaccines showing higher antigen-specific antibody titers also displaying more pronounced virus neutralization effect.

In conclusion, administration of vaccine candidates [redacted] **BNT162b2**, [redacted] via intramuscular injections weekly for 2 or 3 administrations to male and female Wistar Han rats was tolerated without evidence of systemic toxicity and produced nonadverse inflammatory changes at the injection sites and the draining lymph nodes, increased haematopoiesis in the bone marrow and spleen, and clinical pathology changes consistent with an immune response or inflammation in the injection sites. The findings in this study are reversible, consistent with those typically associated with the intramuscular administration of antigens and/or LNPs.

[redacted]
Study Director

17 Sep 2020
Date

2. GENERAL INFORMATION

2.1 Aim of study

The aim of the study was to obtain information on the toxicity of four vaccines based on three LNP-formulated RNA vaccine platforms encoding for viral proteins administered once weekly by intramuscular administration to rats and to assess the reversibility of any effect after a 3-week recovery period.

2.2 Duration of study

- 5 to 11 adaptation days
- 17 test days for groups 1 to [redacted] and group 7
- 10 test days for group [redacted]
- 3 additional weeks for the animals scheduled for the recovery period

2.3 Test items and control

2.3.1 Control (for group 1)

Designation	Buffer (PBS/300 mM Sucrose)
Batch no.	090320
Receipt no.	69570 (one vial with a gross weight of 66.76 g)
Date of receipt	13 March 2020
Characteristics	Liquid
Storage conditions	At +2°C to +8°C
Stability/Retest date	No data available to [redacted] No Certificate of Analysis was available to [redacted]
Retention sample	Stored in [redacted] archives.

[redacted]

[redacted]

2.3.4 Test item 4 (for group 7)

Designation	"RBP020.1" (BNT162b2)
Designation	LNP formulated modRNA encoding the RBD subunit of SARS-CoV-2 S protein ("BNT162b - 2")
Batch no.	CoVVAC/160320

Receipt no.	69580 (100 vials with 500 µL)
Date of receipt	20 March 2020
Characteristics	Frozen liquid (at receipt at [redacted]
Storage conditions	At -70°C or colder
Stability/Retest date	September 2020
Concentration	554 µg RNA/mL For further details see the Certificate of Analysis in Appendix 1.
Retention sample	Stored in [redacted] archives.

[redacted]

[redacted]

2.4 Identification of the test items

After receipt at [redacted] the test items were inspected. Batch numbers, amounts, and characteristics (colour and consistency) were determined and compared with information given by the Sponsor (see text table below). Identification sheets were filed with the raw data.

Text table 2-1: Identification of the test item

Test item / Control	Parameter	[redacted] identification#	Sponsor identification##
Control: Buffer (PBS/300 mM Sucrose)	colour consistency	clear liquid	white to off-white suspension
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
BNT162b2	colour consistency	slightly turbid liquid	white to off-white suspension
[redacted]	[redacted]	[redacted]	[redacted]

Identified before usage, in thawed condition, at room temperature.
According to Certificates of Analysis, for the thawed conditions of the test items.

No further identification was performed by [redacted]

2.5 Sponsor / Test Facility / Responsible personnel

Sponsor [redacted]

Sponsor's contact person [redacted]

Test Facility [redacted]

Branch facility [redacted]

Study Director Until 31 May 2020 [redacted]

As of 01 June 2020 [redacted]

Deputy Study Director [redacted]

[redacted]

2.6 Rules and regulations

2.6.1 Good Laboratory Practice

The study was performed in compliance with the 'Good Laboratory Practice' regulations (see the Statement of Compliance on page 5 and the enclosed 'GLP Certificate of the Test Facility [redacted] in Appendix 3).

2.6.2 Standard Operating Procedures and staff safety

Standard Operating Procedures (SOPs)	All work was carried out according to Standard Operating Procedures which were followed for all stages of the study. The SOPs could be inspected in those divisions, which were engaged in the study and in the Quality Assurance Unit (QAU).
Staff safety	The standard safety precautions operating within the department were applied to this study.

2.6.3 Archiving and storage

Archives of data and specimens

During the study

All data generated at the Branch Facility:

In the depot

[redacted]

All remaining data:

[redacted]

After reporting

The final report and the amendment no. 1 to final report will be archived by the Sponsor.

A copy of the final report, the amendment no. 1 to final report and all specimens, written raw data, and other study-related documents listed in the Study Plan section 'Study Materials to be Archived' are stored in the [redacted] archives. The duration of storage (15 years) will be in compliance with the GLP regulations as stated in the German Chemicals Act ("Chemikalien-gesetz").

All archived study materials will be destroyed after the 15-year GLP storage period unless the Sponsor requests otherwise.

Upon expiry of the 15-year archiving period, the Sponsor will be given three weeks' notice before the study documentation and samples are destroyed by [redacted]. The Sponsor must respond within the 3-week notification period if testing documentation is to be transferred to the Sponsor (shipping fees will be billed). If [redacted] does not receive any response from the Sponsor, the study documentation and samples will be destroyed.

To avoid any doubt, it is the responsibility of the Sponsor to provide [redacted] with a valid contact. The archived documentation and samples will be destroyed without Sponsor notification after 15.5 years of archiving if [redacted] cannot contact the Sponsor because

- a valid Sponsor contact has not been provided
  or
- the Sponsor company no longer exists
  or
- [redacted] has not been advised of the legal successor.

Three months after the issuance of the Final Study Report, any samples or aliquots not listed in the Study Plan section 'Study Materials to be Archived' and still remaining at [redacted] will be listed. This list will be forwarded to the Sponsor who will decide which of these samples or aliquots will be dispatched and which samples or aliquots will be destroyed at [redacted]

2.7 Study dates

Code number of the study in the raw data: 38166

Start of study

Date of Study Plan: 16 March 2020

Study Plan amendments: No. 1, dated 17 March 2020
No. 2, dated 23 March 2020
No. 3, dated 25 March 2020
No. 4, dated 03 April 2020
No. 5, dated 06 April 2020
No. 6, dated 08 April 2020
No. 7, dated 20 April 2020
No. 8, dated 29 May 2020
No. 9, dated 18 June 2020

Text table 2-2: In-life Schedule - Study dates

Animals	First administration	End of in-life period
All main study animals of groups 1, [redacted]	17 March 2020	02 April 2020
All recovery animals of groups 1, [redacted]	17 March 2020	23 April 2020
All main study animals of groups [redacted] and 7	23 March 2020	08 April 2020
All recovery animals of groups [redacted] and 7	23 March 2020	29 April 2020
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
Three erroneously treated animals (non-GLP)	23 March 2020	26 March 2020
All satellite animals of groups 1, [redacted]	24 March 2020	09 April 2020
All satellite animals of groups [redacted] and 7	14 April 2020	30 April 2020
[redacted]	[redacted]	[redacted]

Date of Final Report: 01 July 2020

Date of Amendment No. 1 to Final Report: 17 September 2020

2.8 Study Plan deviations

The study was conducted in accordance with the Study Plan and 9 Study Plan amendments. There was no major deviation from the Study Plan and the Study Plan amendments. However, the following minor deviations were noted:

Animals

A few male and female animals exceeded the allowed body weight range of 10% of the mean weight for each sex at the time of selection. This deviation was due to the limited availability of animals that fully met the required body weight criteria from the breeder.

Two (2) of the 10 delivered female satellite animals designated for blood sampling via the femoral vein catheter showed inflammations at the vascular access button. Hence, these animals were excluded from the study. However, 9 of the 10 animals originally delivered were needed to start dosing as scheduled. Therefore, a female spare animal from the main study animals that was not equipped with a femoral vein catheter was inserted into the satellite group (no. 215). Blood sampling was performed by means of retrobulbar vein puncture from this animal.

Animal housing

A malfunctioning of the air conditioning system caused relative humidity values in the animal room that temporarily fell slightly below the lower admissible limit of 40% on a few test days. The room temperature did not exceed the maximum range during the study period.

[redacted]

Organ weights

The spleen of the female animal no. 144 was not weighed during necropsy.

These minor deviations that were not covered by Study Plan amendments did not affect the validity and integrity of the scientific results obtained during the study.

3. MATERIALS AND METHODS

3.1 Animals

Wistar Han rats supplied by [redacted] were used in this study. The satellite animals were supplied with a pre-implanted femoral vein catheter for repeated blood sampling.

An initial health check was performed upon delivery of the animals. Only animals free of signs of illness were selected for the study.

The animals were allocated to the test groups based on body weight by means of a computerized randomization program (see Section 3.7). Animals with a body weight at the extremes of the weight distribution, if any, were excluded and replaced by healthy spare animals. No replacements occurred after the first dose had been administered.

Test species / Strain / Stock	Rat / Wistar / Crl:WI(Han) All satellite animals (except for no. 215, see Section 2.8) were equipped with a vascular access button to the femoral vein by the breeder.
Breeder	[redacted]
Number and sex of animals	255 animals (129 males and 126 females) Main study (MS) 143 animals (70 + 3 males and 70 females) [redacted] Recovery period (RP) 70 animals (35 males and 35 females) In addition, 20 spare animals (10 males and 10 females) were available for possible replacement. Three of the male spare animals were used to replace the erroneously dosed animals (see above, and Text table 3-2). One of the female spare animals was used as a satellite animal (no. 215, see Section 2.8).

	Satellite animals (SA) 42 animals (21 males and 21 females) In addition, 6 spare satellite animals (3 males and 3 females) were available for possible replacement. The satellite animals were supplied in two separate shipments (10 animals per sex for groups 1[redacted] and 14 animals per sex for groups [redacted]/7) to[redacted] Two (2) of the 10 animals of the shipment for groups 1[redacted] showed inflammations at the vascular access button and were excluded from the study. As 9 animals were needed to start dosing as scheduled, one female spare animal from the main study animals not equipped with a vascular access button was used as satellite animal (see further above).
Age (at 1st dosing)	Groups 1, [redacted] Males and females: 54 days Groups [redacted] and 7: Males and females: 60 days
Body weight (at 1st dosing)	Males: 252.8 to 343.9 g Females: 188.3 to 267.3 g
Selection of species	The rat is a commonly used rodent species for toxicity studies. It can receive [redacted] and develops an immune response similar to the expected human response after vaccination.
Identification of animals	After randomisation, each rat received a continuous number on the tail, either by tattoo or marker. Additionally, the animal cages were labelled with study number, animal ID number, sex, type of study, route of administration, and treatment group.
Adaptation period	Groups 1, [redacted] 5 days Groups [redacted] and 7: 11 days

3.2 Housing and feeding

3.2.1 Diet

A certified commercial pellet diet (ssniff® R/M-H V1534, ssniff Spezialdiäten GmbH, 59494 Soest, Germany; see Appendix 2: 'Composition of the Diet') served as food. The food was offered *ad libitum*. Food residue was removed and weighed.

Periodic analysis of the food for contaminants based on EPA/USA[2] is conducted at least twice a year by LUFA-ITL[3] (see Appendix 2: 'Limitation for Contaminants in the Diet'). Certificates of analysis of the composition and for contaminants were provided by the manufacturer and were included in the raw data.

3.2.2 Drinking water

Drinking water was offered *ad libitum*.

Samples of drinking water are taken by Wasserwerk Wankendorf and periodic analyses are performed by LUFA-ITL according to the 'Deutsche Trinkwasser-verordnung 2001' [German Regulations on Drinking Water 2001][4] (see Appendix 2: 'Limitation for Contaminants in the Drinking Water').

In addition, drinking water samples taken at [redacted] are analysed by LUFA-ITL once a year for means of bacteriological investigations according to the 'Deutsche Trinkwasserverordnung 2001, Anlage 1' [German Regulations on Drinking Water 2001, Addendum 1].

3.2.3 Housing

The animals were kept singly in MAKROLON cages (type III plus) with a basal surface of approximately 39 cm × 23 cm and a height of approximately 18 cm at a room temperature of 22°C ± 3°C (maximum range) and a relative humidity of 55% ± 10% (maximum range). Deviations from the maximum range caused for example during cleaning procedures were dealt with in SOPs.

The rooms were lit (about 150 lux at approx. 1.5 meters room height) and darkened for periods of 12 hours each.

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[2] EPA/USA, Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, Federal Register 44, 27334 - 27375, May 1979.

[3] Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Institut für Tiergesundheit und Lebensmittelqualität GmbH, 24107 Kiel, Germany.

[4] Version from 02 August 2013, revised on 20 December 2019.

Granulated textured wood (Granulat A2, J. Brandenburg, 49424 Goldenstedt, Germany) was used as bedding material for the cages. The cages were changed and cleaned once a week.

Periodic analysis of the bedding material for contaminants based on EPA/USA is conducted at least once a year by LUFA-ITL (see Appendix 2: 'Limitation for Contaminants in the Bedding Material').

3.3 Dose selection

The dose levels for this study had been selected in agreement with the Sponsor based on the [redacted].

3.4 Test item preparation

The test items were delivered ready-to-use.

The LNP suspensions for dosing were used within 6 hours after thawing.

Protocol:

1. The test item vials required were thawed by removing from the -80°C ±8°C storage and allowed to warm to room temperature (approximately 30 minutes).
2. Each vial was mixed by gently inverting three times. The vials were neither mixed vigorously nor vortexed.
3. The vial's flip cap was flipped off.
4. The needle was inserted through the stopper into a vial and the appropriate volume per animal withdrawn. The procedure was repeated until the total needed volume per animal had been obtained. A new syringe (including needle) was used for each animal.
5. After the last administration of a day, any remaining volumes of the thawed test items were discarded; remnants were not re-frozen or re-used.

3.5 Test item formulation analysis

As the test items were delivered ready-to-use no formulation analysis was required.

3.6 Administration

Route of administration	Intramuscular (i.m.) administration into the *Musculus biceps femoris* using a Microfine+ Syringe 0.5 mL, 0.33 mm (29G) × 12.7 mm (BD, 324824).

Frequency of administration

Groups 1, [redacted] and 7:
On test days 1, 8 and 15; in total 3 administration days at one-week intervals per animal.

[redacted]

Erroneously treated animals:
Single dose (2 administration sites) on test day 1

Administration volume

Groups 1 to 7:

Text table 3-1: Administration volume

Group	Number of administration sites	Administration volume per administration day [µL]	
		Per site	Per animal
1	2	100	200
[redacted]			
7	2	100	200

Erroneously treated animals:
[redacted] µL/administration site; 2 administration sites
In total [redacted] µL/animal/administration day

Dosages

Groups 1 to 7:
Group 1: Control (200 µL Buffer/animal)

[redacted]

See the text table in Section 3.7 for details.

Selection of route of administration

According to clinical use. The intramuscular route is the anticipated route for human exposure to the test item.

3.7 Group size and dose levels

The animals were allocated to 7 test groups by means of a computer generated randomisation program[5] and treated as given in the text table below.

Text table 3-2: Group distribution and dosing scheme

Group	Dose level [µg/animal] (Test item / Control)	Number and sex of animals MS + RP + SA	Animal no.		
			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
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
7	100 (BNT162b2)	10 + 5 + 3 m 10 + 5 + 3 f	181 - 190 196 - 205	191 - 195 206 - 210	247 - 249 250 - 252
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

m male
f female
MS Main study
RP Recovery period
SA Satellite animals for cytokine analysis (except animals nos. 253 to 255)
[redacted]

The satellite animals of groups 1 to 7 were used for blood sampling only (see Section 3.8.7.5). Following the last blood sampling, these animals were sacrificed but not dissected.

[5] Provantis® Integrated preclinical software, version 10.2, Instem LSS Ltd, Stone, Staffordshire ST15 0SD, United Kingdom.

[redacted]

3.8 Observations

All in-life examinations described in the following subsections pertain only to the main study and recovery animals, i.e. the satellite animals were excluded from these examinations.

Dated and signed records of all activities related to the day-to-day running and maintenance of the study within the animal unit as well as to the group observations and examinations outlined in the Study Plan were recorded in appropriate documentation. In addition, observations related to individual animals were made throughout the study and recorded.

The following sections describe the observations made during the course of the study.

3.8.1 Clinical signs

The animals were observed individually before and after dosing at each time of dosing for any signs of behavioural changes, reaction to treatment or illness.

In addition, the animals were checked regularly throughout the working day from 7:00 a.m. to 3:45 p.m. (i.e. starting approximately at 7:00 a.m., 9:00 a.m., 11:00 a.m., 1:00 p.m. and 3:00 p.m.). On Saturdays and Sundays, the animals were checked regularly from 7:00 a.m. to 11:00 a.m. with a final check performed at approximately 3:30 p.m. (i.e. starting at approximately 7:00 a.m., 9:00 a.m., 11:00 a.m., and 3:00 p.m.).

Cageside observations included skin/fur, eyes, mucous membranes, respiratory and circulatory systems, somatomotor activity and behaviour patterns. The onset, intensity and duration of any signs observed were recorded.

Dated and signed records of appearance, change and disappearance of clinical signs of individual animals were maintained on clinical history sheets.

Special attention was paid to the local tolerance at the injection sites (see Section 3.8.3 for details on the observation of erythema/eschar, oedema, induration/hardening).

3.8.2 Mortality

Further checks were made early in the morning and again in the afternoon of each working day to look for dead or moribund animals. On Saturdays and Sundays, a similar procedure was followed with a final check at approximately 3:30 p.m.

These provisions allowed for recording of premortal symptoms in detail and for performing post mortem examinations as soon as possible after exitus. However, no premature deaths occurred and no premature sacrifice was necessary.

3.8.3 Local tolerance

The local tolerance of the test item at the injection site was recorded for all main study and recovery animals at the following times:

- 4 h after each injection
- 24 h after each injection
- 48 h after each injection
  As irritations were still present at 48 h after injection, the observations of the respective animal was extended to every 48 h until the irritation had resolved (i.e. 96 h p.a. and 144 h p.a. if necessary).

The injection sites were assessed for

- erythema and eschar formation
- oedema formation
- induration/hardening following palpation

The reactions were scored with a grading similar to that based on DRAIZE (Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, Association of Food and Drug Officials of the United States, Austin, Texas, 1959) as given in detail in the text tables following on the following page.

Text table 3-3: Grading of erythema and eschar formation

Erythema and eschar formation	**Value**
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) or eschar formation (injuries in depth) preventing erythema reading	4

Text table 3-4: Grading of oedema formation

Oedema formation	**Value**
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approx. 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

Text table 3-5: Grading of induration/hardening

Grade of induration/hardening	**Value**
No induration/hardening	0
Very slight induration/hardening (barely perceptible)	1
Slight induration/hardening	2
Moderate induration/hardening	3
Severe induration/hardening	4

If there were two injection sites per animal (left/right side), individual values for each of the two injection sites were only recorded in case the gradings of the sites were different (e.g. '0/1'). If the gradings of the two injection sites were identical only one scoring was recorded.

In addition, any signs of pain were recorded as general observations of local tolerance.

3.8.4 Body weight

The body weight of each rat was recorded at the following times:

- at group allocation
- prior to each administration: on test days 1, 8, and 15 (if applicable)
- one day after each administration: on test days 2, 9, and 16 (if applicable)
- twice weekly during the recovery period
- at autopsy (i.e. during the process of necropsy, i.e. after fasting overnight and exsanguination, see Section 3.8.10.1 for details)

3.8.5 Food and drinking consumption

The quantity of food left by individual animals was removed, weighed, and recorded on a weekly basis throughout the experimental period. The residue was discarded.

The food intake per animal (g/animal/week) was calculated using the total amount of food given to and left by each rat in each group on completion of a treatment week. Weekly mean values were calculated for individual animals.

The relative food consumption (in g/kg b.w./day) was calculated as follows:

$$\text{Relative food consumption (g/kg b.w./day)} = \frac{\text{Total food given (g) - Total food left (g)}}{\text{Number of animal days}^{\#} \times \text{Body weight (kg)}}$$

[#] The term 'animal days' counts one animal day for each animal alive for a whole day; it is assumed that on the day of death an animal does not eat.

The drinking water consumption was monitored daily by visual appraisal throughout the study. The consumption was not quantified.

3.8.6 Body temperature

The body temperature was determined using an anal probe at the times stated in the text table below.

Text table 3-6: Time points for body temperature measurement

Test day	Time points for body temperature measurement relative to dosing	Groups or animal number	Main study animals	Recovery animals
1	4 hours after 1st injection	1 to 7	X	X
2	24 hours after 1st injection	1 to 7	X	X
[redacted]	[redacted]	[redacted]		
8	4 hours after 2nd injection	1 to 7	X	X
9	24 hours after 2nd injection	1 to 7	X	X
15	1 week after last administration	[redacted] and 7		X
15	4 hours after 3rd injection	1 to [redacted]	X	X
16	24 hours after 3rd injection	1 to [redacted]	X	X
[redacted]	[redacted]	[redacted]		
22	1 week after last administration	1 to [redacted]		X
22	2 weeks after last administration	[redacted] and 7		X
29	2 weeks after last administration	1 to [redacted]		X
29	3 weeks after last administration	[redacted] and 7		X
36	3 weeks after last administration	1 to [redacted]		X

[redacted]

[redacted]

3.8.7 Laboratory examinations

Blood samples were taken from the retrobulbar venous plexus under isoflurane anaesthesia from animals fasted overnight. The blood samples were collected into tubes as follows:

EDTA anticoagulant (whole blood).....for haematological investigations
Citrate anticoagulant (plasma)..... for coagulation tests
Li-Heparin anticoagulant (plasma) .....for clinical chemistry tests
Serum (no anticoagulant) .....for acute phase proteins and dose exposure

The male animal no. 130 died during the blood withdrawal on test day 17. The blood sample intended for clinical chemistry tests was obtained by heart puncture from this animal. As a result, the plasma levels of phosphate and potassium, and the enzyme activities of ASA and LDH were far beyond the normal range for these parameters. Therefore, these data were excluded from statistical analysis (marked by 'E!' in Table 1-2).

3.8.7.1 Haematology

The blood samples were obtained as follows:

- On test day 4:	The first 5 surviving main study animals per sex and group and all recovery animals (n = 10 per group).
- At main study termination (on the day of dissection):	All main study animals (n = 10 per group).
- At the end of the recovery period (on the day of dissection):	All recovery animals (n = 5 per group).

The haematological parameters listed in the text table below were determined.

Text table 3-7: Haematological parameters

Parameter	Unit	Instrument
Haemoglobin content (HGB)	mmol/L	ADVIA™ 120 Siemens Diagnostics GmbH 35463 Fernwald Germany
Erythrocytes (RBC)	$10^6/\mu L$	
Leucocytes (WBC)	$10^3/\mu L$	
Reticulocytes (Reti), relative	%	
Reticulocytes (Reti), absolute	$10^3/\mu L$	
Platelets (PLT)	$10^3/\mu L$	
Haematocrit value (HCT)	%	
Differential blood count (relative)[#]	%	
Differential blood count (absolute)[#]	$10^3/\mu L$	
Mean corpuscular volume (MCV)	fL	
Mean corpuscular haemoglobin (MCH)	fmol	
Mean corpuscular haemoglobin concentration (MCHC)	mmol/L	
Mean platelet (thrombocyte) volume (MPV)	fL	
Relative volume of thrombocytes / Plateletcrit (PCT)	%	
Platelet distribution width (PDW)	%	
Red cell distribution width (RDW)	%	
Mean platelet component (MPC)	g/dL	

[#] Neutrophilic, eosinophilic and basophilic granulocytes, lymphocytes, and monocytes. Large unstained cells were simultaneously quantified during measurement of the differential blood count.

Following the haematological examinations using the ADVIA system, blood smears were prepared from all samples, dried, and stained as given in the text table on the following page.

Text table 3-8: Staining of blood smears

Dissection	Group	Number of stainings	
		Pappenheim	Brilliant Cresyl blue
Test day 4	Groups 1, [redacted]	1	0
	Groups [redacted] and 7	1	0
Main study	Groups 1, [redacted]	1	1
	Group [redacted]	1	1
	Groups [redacted] and 7	1	1
Recovery	Groups 1, [redacted]	1	1
	Group [redacted]	1	1
	Groups [redacted] and 7	1	1

The stained blood smears may be evaluated, if requested by the Sponsor (details are to be stated in a Study Plan amendment). So far, no evaluation of blood smears was performed by [redacted]

3.8.7.2 Coagulation

The blood samples were obtained as follows:

- At main study termination (on the day of dissection): All main study animals (n = 10 per group).
- At the end of the recovery period (on the day of dissection): All recovery animals (n = 5 per group)

The coagulation parameters listed in the text table below were determined.

Text table 3-9: Coagulation parameters

Parameter	Unit	Instrument
Prothrombin time (PT)	sec	Amax Destiny Plus™ Tcoag Deutschland GmbH 32657 Lemgo, Germany
Activated partial thromboplastin time (aPTT)	sec	
Fibrinogen	mg/dL	

3.8.7.3 Clinical chemistry

The blood samples were obtained as follows:

- On test day 4: The first 5 surviving main study animals per sex and group and all recovery animals (n = 10 per group).
- At main study termination (on the day of dissection): All main study animals (n = 10 per group).
- At the end of the recovery period (on the day of dissection): All recovery animals (n = 5 per group).

The clinical chemistry parameters listed in the text table below were determined.

Text table 3-10: Clinical chemistry parameters

Parameter	Unit	Instrument / Method
Albumin	g/L plasma	KONELAB 30i (see below)
Globulin	g/L plasma	By subtraction
Albumin/globulin ratio	(non-dimensional)	By calculation
Bilirubin (total)	µmol/L plasma	KONELAB 30i Thermo Fisher Scientific 63303 Dreieich Germany
Cholesterol (total)	mmol/L plasma	
Creatinine	µmol/L plasma	
Glucose	mmol/L plasma	
Phosphate	mmol/L plasma	
Protein (total)	g/L plasma	
Urea (in blood)	mmol/L plasma	
Triglycerides	mmol/L plasma	
Calcium	mmol/L plasma	
Chloride	mmol/L plasma	
Potassium	mmol/L plasma	
Sodium	mmol/L plasma	
Alanine aminotransferase (ALAT)	U/L plasma	
Alkaline phosphatase (aP)	U/L plasma	
Aspartate aminotransferase (ASAT)	U/L plasma	
Lactate dehydrogenase (LDH)	U/L plasma	
Creatine kinase (CK)	U/L plasma	
Gamma-glutamyltransferase (Gamma-GT)	U/L plasma	

3.8.7.4 Analysis of acute phase proteins

Blood samples were obtained as follows:

- On test day 4: The first 5 surviving main study animals per sex and group and all recovery animals (n = 10 per group).
- At main study termination (on the day of dissection): All main study animals (n = 10 per group).
- At the end of the recovery period (on the day of dissection): All recovery animals (n = 5 per group).

In order to obtain approximately 4 × 75 µL serum per animal and sampling time, approx. 0.7 mL whole blood per animal and sampling time were collected in serum separator tubes (Sarstedt AG & Co., Germany). The blood samples were allowed to clot at room temperature for at least 30 minutes, and centrifuged afterwards in order to obtain serum. Immediately after centrifugation, the serum was divided into aliquots and frozen at -20°C ±2°C until analysis at [redacted] using commercial ELISA test kits purchased from Abcam PLC, Cambridge, United Kingdom (see text table below) and a Tecan Sunrise microplate reader (Tecan Deutschland GmbH, 74564 Crailsheim, Germany).

Text table 3-11: Parameters of acute phase protein analysis

Acute phase protein	Matrix	Sample volume	Number of aliquots (aliquot volume)	Storage temperature	Method
Alpha1-acid glycoprotein	Serum	150 µL	2 (75 µL)	-20°C ±2°C	Rat Alpha 1 Acid Glycoprotein / AGP ELISA Kit (ab157729, lot no. GR3235007-3)
Alpha2 macroglobulin	Serum	150 µL	2 (75 µL)	-20°C ±2°C	Rat alpha 2 Macroglobulin ELISA Kit (ab157730, lot nos. GR3322797-1, GR3322797-3, and GR3322797-4)

3.8.7.5 Cytokine analysis

Blood samples for cytokine analysis were taken from the femoral vein catheter of all satellite animals at the times given in the text table below.

Text table 3-12: Blood sampling schedule for cytokine analysis

Test day	Sampling time relative to dosing	Animal numbers of satellite animals used							Number of samples/ aliquots
		Group 1[#]	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	
1	Prior to 1st dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	247 to 252	42/84
1	6 h post 1st dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	247 to 252	42/84
8	Prior to 2nd dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	247 to 252	42/84
8	6 h post 2nd dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	247 to 252	42/84
10	48 h post 2rd dosing						[redacted]		6/12
15	Prior to 3rd dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]		247 to 252	36/72
15	6 h post 3rd dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]		247 to 252	36/72
17	48 h post 3rd dosing	211 to 216	[redacted]	[redacted]	[redacted]	[redacted]		247 to 252	36/72
								Total number of samples / aliquots:	**282/564**

[#] Blood sampling from the female animal no. 215 was performed by means of retrobulbar vein puncture (refer to Section 2.8 and Section 3.1).

Sufficient whole blood was collected from the animals in order to obtain at least 2×75 µL serum per animal and sampling time. The serum samples were frozen, stored and analysed as given in Text table 3-15 below.

Text table 3-13: Parameters of cytokine analysis

Cytokine	Matrix	LLOQ [pg/mL]	Sample volume	Number of aliquots (aliquot volume)	Storage temperature	Method
IFN-γ TNF-α IL-1-β IL-6 IL-10	Serum	4.0 7.1 12.6 3.0 9.9	150 µL	2 (75 µL)	-20°C ± 2°C	Cytometric bead array (ProcartaPlex) using a Cytomics FC 500 flow cytometer (Beckman Coulter GmbH, 47704 Krefeld, Germany)

LLOQ lower limit of quantification

3.8.7.6 Urinalysis

Urine samples were collected from animals at the following time points:

- At main study termination (on the day of dissection): All main study animals (n = 10 per group).
- At the end of the recovery period (on the day of dissection): All recovery animals (n = 5 per group).

The urine was collected in a URIMAX funnel cage for 16 hours. The collection of urine was terminated immediately prior to the blood withdrawals for haematological and clinical chemistry examinations. The parameters measured and the methods used are given in the text table below.

Text table 3-14: Urinary parameters

Parameter	Unit	Instrument
Volume	mL	Graduated vessel
pH	n/a	Digital pH meter (type WTW InoLab pH 720)
Specific gravity	g/mL	Kern Refractometer (type ORA 2PA), sample compared with water (nominal value of 1.000)

In addition, the tests given in the text table below were performed using qualitative indicators (Combur 9® Test, Roche Diagnostics GmbH, 68305 Mannheim, Germany) of analyte concentration:

Text table 3-15: Analytes of qualitative urinalysis

Parameter	Reporting convention					
	Unit	Semi-quantitative determination level				
Protein	g/L	neg	0.3	1	5	
Glucose	mmol/L	normal	2.8	5.5	17	55
Bilirubin	-	neg	+	+ +	+ + +	
Urobilinogen	µmol/L	normal	17	70	140	200
Ketones	-	neg	+	+ +	+ + +	
Haemoglobin (Hb, approx. values)	ery/µL	neg	10	25	50	250
Nitrite	-	neg	pos			

neg = negative
pos = positive
ery = erythrocyte count

\+ 'Small amount' of analyte
+ + 'Moderate amount' of analyte
+ + + 'Large amount' of analyte

A microscopic examination of urine samples was carried out by centrifuging samples and spreading the resulting deposit on a microscope slide. The deposit was examined for the presence of the following parameters:

- Epithelial cells
- Leucocytes
- Erythrocytes
- Organisms
- Further constituents (i.e. sperm, casts)
- Crystalluria

The frequency of the above parameters in the centrifugal deposit was recorded as follows:

0	None found in any field examined
+	Few in some fields examined
+ +	Few in all fields examined
+ + +	Many in all fields examined

The colour and the turbidity of the urine were examined visually.

3.8.8 Blood sampling for dose exposure

In order to obtain serum samples for dose exposure examination (10 aliquots of approximately 100 µL each per animal), blood was withdrawn from the retrobulbar venous plexus under isoflurane anaesthesia from animals fasted overnight as follows:

- At main study termination (on the day of dissection): All main study animals.
- At the end of the recovery period (on the day of dissection): All recovery animals

In total 209 samples (2074 aliquots) were collected. [redacted] Further, for a few animals, the blood volume sampled was not sufficient for 10 aliquots of 100 µL serum each but yielded only 7 to 9 aliquots.

After collection of sufficient whole blood in serum separator tubes (Sarstedt®, Germany), the blood samples were allowed to clot for at least 30 minutes before centrifugation. Immediately after centrifugation, the serum was frozen and stored at -80°C ±8°C until shipment for analysis.

The samples were labelled with the study number, species, animal number, type of sample, purpose (dose exposure), aliquot no., group number, test day and date.

Following advance notice by e-mail (to: [redacted] and [redacted], the dose exposure samples were dispatched on dry ice via courier as given in the text table below.

Text table 3-16: Shipping schedule of dose exposure samples

Shipped by [redacted]	Delivered to the Sponsor	Animals	Aliquots included	Consignee
06 Apr 2020	07 Apr 2020	Groups 1, [redacted] Main Study N = 80	Aliquots 1 to 10# (n = 790)	[redacted]
09 Apr 2020	09 Apr 2020	Groups [redacted] 7 Main study N = 60	Aliquots 1 to 10# (n = 586)	
30 Apr 2020	30 Apr 2020	Groups 1 to 7 Recovery N = 70	Aliquots 1 to 10# (n = 698)	

As far as available. For a few animals, less than 10 aliquots (but at least 7 aliquots) per sampling were available as the blood volume sampled was not sufficient to yield 10 aliquots of 100 µL serum each.

[redacted]

The samples were analysed for immunogenicity of the test items by [redacted] under the responsibility of the Sponsor. An analytical report was forwarded to [redacted] (see Section 4.12 and Appendix 4).

3.8.9 Ophthalmological and auditory examinations

Examinations were performed on all main study and recovery animals before first dosing and at the end of the dosing period (groups 1 [redacted] and 7: test day 16, [redacted] and for all recovery animals at the end of the recovery period (groups 1[redacted] and 7: test day 37, [redacted]

The eyes were examined with a HEINE ophthalmoscope. After examination of the pupillary reflex, mydriasis was produced by instillation of STULLN® eye drops (Ankerpharm GmbH, 07407 Rudolstadt, Germany) onto the cornea.

The following ocular structures were examined:

- Adnexa oculi (i.e. lids, lacrimal apparatus), conjunctiva
- Cornea, anterior chamber
- Lens, vitreous body, fundus (retina, optic disc)

The auditory acuity was checked with a simple noise test.

3.8.10 Pathology and histopathology

3.8.10.1 Necropsy

For groups 1 [redacted] and 7, necropsy was scheduled for test day 17 (approximately 48 hours after the last administration) for the main study animals and for test day 38 for all animals allocated to the recovery period.

[redacted]

The animals were sacrificed and dissected following a randomization scheme.

The animals were euthanized by carbon dioxide (CO_2) inhalation, exsanguinated by cutting the aorta abdominalis, weighed, dissected, and inspected macroscopically under the direction of a pathologist.

All superficial tissues were examined visually and by palpation. The cranial roof was removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral midline incision and skin reflection, all subcutaneous tissues were examined. The condition of the thoracic viscera was noted with due attention to the thymus, lymph nodes and the heart.

The abdominal viscera were examined before and after removal; the urinary bladder was examined externally and by palpation. The gastro-intestinal tract was examined as a whole, and stomach and caecum were incised and examined. The lungs were removed and all pleural surfaces were examined under suitable illumination. The liver and the kidneys were examined. Any abnormalities in the appearance and size of the gonads, adrenal glands, uterus, intraabdominal lymph nodes and accessory reproductive organs were recorded.

None of the satellite animals was dissected and examined macroscopically as none of these animals had deceased or was prematurely sacrificed.

The erroneously administered animals (see Section 3.7) were not dissected.

[redacted]

The organs listed in the text table below were weighed before fixation.

Text table 3-17: Weighed organs

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 (one cervical, one mesenteric)	

The paired organs were identified as left or right and weighed individually.

Organ/body weight ratios were calculated (using the body weight at autopsy obtained after exsanguination at necropsy) and are presented as relative organ weights (in g/kg b.w.).

3.8.10.2 Organ preservation

The organs or parts of organs of all animals listed in the text table on the following page were fixed in 7% neutral buffered formalin, except for the eyes which were fixed in Davidson's solution, and the testes which were fixed in modified Davidson's solution for optimum fixation.

Text table 3-18: Tissues collected for preservation

Tissues preserved for histopathology	
Adrenal gland *(2)*	Mammary gland
# Animal ID	Muscle *(skeletal, leg)*
Aorta abdominalis	Nerve *(sciatic)*
# Body cavity, nasal	# Nerve *(tibial, 2)*
Bone *(os femoris with joint)*	Oesophagus
Bone *(sternum)*	Ovary *(2)*
Bone marrow *(os femoris)*	Oviducts *(2)*
Brain *(cerebrum, cerebellum, brain stem)*	Pancreas
Caecum	Parathyroids
# Clitoral gland *(2)*	Pituitary
Epididymis *(2)*	# Preputial gland *(2)*
Eye with optic nerve *(2)*	Prostate
# Ganglion, dorsal root, lumbar	Salivary glands *(mandibular, parotid, sublingual)*
Gut-associated lymphoid tissue	
Harderian gland *(2)*	Seminal vesicle *(2)*
Heart *(left and right ventricle, septum)*	Skin *(left flank*)
Injection sites 1 and 2	Spinal cord *(3 sections)*
Intestine, small *(duodenum, jejunum, ileum, Swiss roll method)*	Spleen
	Stomach
Intestine, large *(colon, rectum)*	Testicle *(2)*
Kidney and ureter *(2)*	Thymus
Lacrimal gland *(extraorbital)*	Thyroid *(2)*
# Larynx	Tongue *(including base)*
Liver *(2 lobes)*	Trachea
Lungs *(with mainstem bronchi and bronchioles)*	# Ureter *(2)*
Lymph node *(1, cervical)*	Urinary bladder
Lymph node *(1, mesenteric)*	Uterus *(including cervix)*
# Lymph node *(2, mandibular)*	Vagina
Lymph node *(1, draining administration site: iliac)*	# Zymbal's gland *(2)*

The tissues marked with the hash sign ('#') were preserved, but not further processed. They may be evaluated if requested by the Sponsor. So far, no evaluation was performed by [redacted]

3.8.10.3 Bone marrow

During dissection, fresh bone marrow was obtained from the os femoris (3 air-dried smears per animal) of the first 5 main study animals per sex and group, and of all recovery animals and stained as given in the text table below.

Text table 3-19: Staining of bone marrow smears

Dissection	Group	Number of stainings	
		Pappenheim	Giemsa
Main study (5 animals per sex and group)	Groups 1, [redacted]	1	0
	Group [redacted]	1	0
	Groups [redacted] and 7	1	2
Recovery (5 animals per sex and group)	Groups 1, [redacted]	1	2
	Group [redacted]	1	2
	Groups [redacted] and 7	1	2

The stained bone marrow smears may be evaluated, if requested by the Sponsor (details are to be stated in a Study Plan amendment). So far, no evaluation of bone marrow smears was performed by [redacted]

3.8.10.4 Histopathology

The organs listed in Section 3.8.10.2, with the exception of the organs marked with the hash sign ('#'), of all main study and recovery animals of all groups were examined histopathologically after preparation of paraffin sections and haematoxylin-eosin staining.

Parathyroids cannot always be identified macroscopically. They were examined microscopically if in the plane of section and in cases they were noted as grossly enlarged.

Blood smears prepared for haematological examination (see Section 3.8.7.1) are available for a possible examination of pathological changes but may be examined and evaluated only depending on necropsy findings and upon agreement with the Sponsor. So far, no examination was performed.

3.8.11 Statistics

All toxicology and pathology data were captured, as far as possible, using the departmental computerized systems (Provantis® Integrated preclinical software, version 10.2, Instem LSS Ltd., Stone, Staffordshire ST15 0SD, United Kingdom). Raw data not fully compatible with the computerized systems were maintained on paper according to appropriate SOPs.

The test item-treated groups [redacted] and 7 were compared to the control group 1. [redacted]

- Haematology and coagulation
- Clinical chemistry
- Urinalysis
- Cytokines
- Acute phase proteins
- Relative and absolute organ weights

The statistical methods described in the text table below were used for the data captured with the Provantis system.

Text table 3-20: Statistical methods

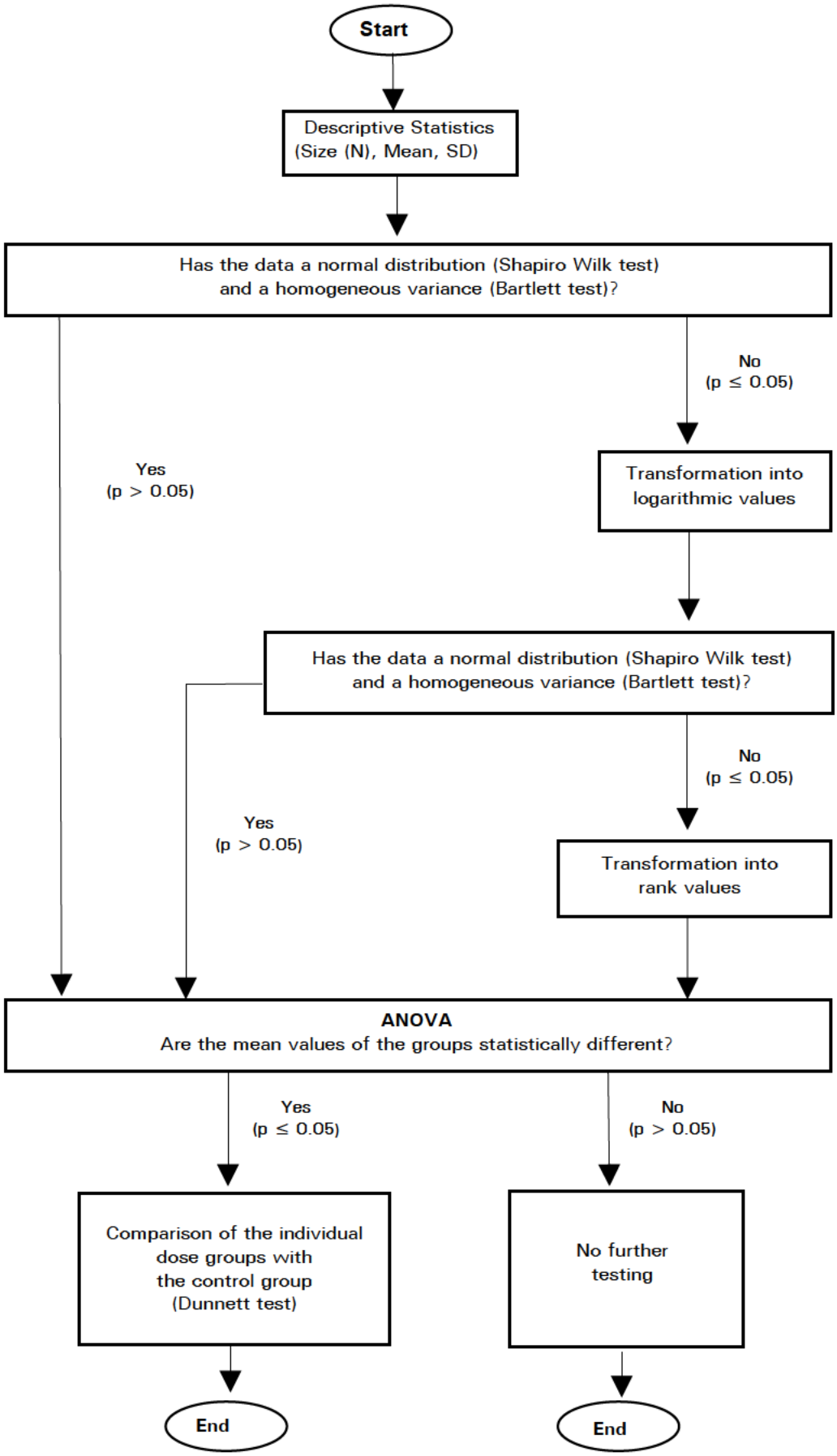
Statistical method	Parameters analysed
Multiple t-test based on DUNNETT, C. W. New tables for multiple Comparisons with a control *Biometrics,* 482-491 (Sept 1964)	Body weight / Food consumption / Haematology and coagulation / Clinical chemistry / Acute phase proteins / Urinalysis / Cytokines / Relative and absolute organ weights ($p \leq 0.05$ and $p \leq 0.01$)
Exact test of R. A. FISHER (if applicable)	Histopathology ($p \leq 0.05$)

The following settings were used for the statistical evaluation of the parametrical values captured by Provantis (see flow chart of decision tree on page 59):

Homogeneity of variances and normality of distribution were tested using BARTLETT's test and SHAPIRO-WILK's test. In case of heterogeneity and/or non-normality of distribution, stepwise transformation of the values into logarithmic or rank values was performed prior to ANOVA. If the ANOVA yielded a significant effect ($p \leq 0.05$), intergroup comparisons with the control group were made by DUNNETT's test (see above).

The statistical procedures were used for all data. Statistically significantly different data are indicated in the tables of Section 5 ('TABLES') of the report.

Flow Chart of Decision Tree of ANOVA and Dunnett's Test Calculation



4. RESULTS

4.1 Local tolerance

[redacted]

[redacted]

[redacted]

BNT162b2 - Group 7

Treatment period

Very slight to severe (very rarely) oedema were noted for all animals following the 1st, 2nd, and/or 3rd injection of **100 µg BNT162b2/animal** on test days 1, 8, and/or 15. All oedema noted after the 1st or 2nd injection had subsided by 96 h p.a. after the respective administration.

In addition, a few female animals also revealed very slight erythema following 24 to 96 h following the 1st or 2nd injection. For individual male and female animals, skin reddening (scored as "severe" erythema) was observed only at 144 h after the 2nd injection, 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**.

Recovery period

Very slight (mostly) to moderate (rarely) oedema were still noted for all animals previously treated with **100 µg BNT162b2/animal** during the early part of the recovery period (following the 3rd injection on test day 15). All local skin reactions had subsided by 336 h p.a. (test day 29).

No abnormalities were noted at the injection site(s) at macroscopic inspection at necropsy at the end of the recovery period.

Histopathological examination of injection sites

Treatment period

The histopathological examination revealed test item-related injection site findings in all groups, characterized by mostly moderate inflammation (up to marked) in males and moderate inflammation in females. The most severe findings were noted consistently in animals administered [redacted] and **100 µg BNT162b2/animal**, [redacted]. The inflammation was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis, at the injection site. Injection site inflammation was associated with mostly moderate oedema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. [redacted] There were no notable injection site findings in control-item administered groups. 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).

Recovery period

Microscopic findings noted at the injection sites were partially or fully resolved at the end of the 3-week recovery phase. A few inflammatory lesions were still noted at the injection sites and the surrounding tissue of some animals.

Microscopic injection site findings correlated with macroscopic findings of thickening, induration, and incrustation.

General observations of local intolerance reactions are given in Table 1-1 (Local tolerance - General observations) and observations of erythema, oedema, indurations, and/or hardenings are listed in Table 1-2 (Local tolerance - Erythema, Oedema, Induration, Hardening).

For detailed listings of histopathological findings at the injection sites, refer to [redacted] Text table 4-26 [redacted] 100 µg BNT162b2/animal, group 7).

4.2 Clinical signs

[redacted]

[redacted] **BNT162b2 - Group 7**

Treatment and recovery period

None of the male and female animals treated intramuscularly with [redacted] **BNT162b2/animal** (group 7) on test days 1, 8, and 15 (3 administrations), [redacted] revealed any test item-related systemic changes in behaviour, external appearance, or consistency of faeces.

A summary of clinical observations is given in Table 2-1 (Clinical Signs - Summary), the individual observations are listed in Table 2-2 (Clinical Signs - Individual Data).

4.3 Mortality

[redacted]

[redacted] **BNT162b2 - Group 7**

Treatment and recovery period

No test item-related deaths were noted for any treatment.

[redacted]

4.4 Body weight

Mean values per group and individual data of body weight, body weight gain, and body weight at autopsy are listed in Table 3-1 (Body Weight - Summary), Table 3-2 (Body Weight - Individual Data), Table 3-3 (Body Weight Gain-Summary), Table 3-4 (Body Weight Gain - Individual Data), and Table 3-5 (Body Weight at Autopsy).

The mean body weight is plotted in Figure 1-1 (male animals) and Figure 1-2 (female animals) on the following page.

The mean body weight gain for the treatment period from [redacted] test day 16 (groups 1 to [redacted] and 7) and for the recovery period from [redacted] test day 16 to test day 37 is shown graphically in Figure 1-3 (male animals) and Figure 1-4 (female animals) on page 72.

The body weight at autopsy at terminal sacrifice (test day 9 for group [redacted] test day 16 for groups 1 to [redacted] and 7) and at recovery sacrifice (test day 30 for group [redacted] test day 37 for groups 1 to [redacted] and 7) is shown graphically in Figure 1-5 (male animals) and Figure 1-6 (female animals) on page 73.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted] [redacted]

[redacted]				
[redacted]	[redacted]			
	[redacted]		[redacted]	
	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted] [redacted]

[redacted]

[redacted]

[redacted]

BNT162b2 - Group 7

Treatment period

Up to test day 8, the male animals treated with **100 µg BNT162b2/animal** (group 7) revealed body weights that were up to 16% *higher* than in the control group due to their older age (+6 days compared to the control animals). A body weight reduction of up to 11.3% was noted compared to the control group on test days 9, 15, and 16 (statistically significant at $p \leq 0.01$ on test days 9 and 16). There was a body weight gain of only approx. 5% for the period from test day 1 to test day 16, which is approx. 32 percentage points lower compared to the control group. The body weight at autopsy was approx. 8% lower compared to the control group on test day 17.

The female animals treated with **100 µg BNT162b2/animal** revealed a reduction of body weight by up to 6.8 % compared to the control group starting on test days 2 to 16 (statistically significant at $p \leq 0.01$ or $p \leq 0.05$ on test days 9 and 16). A body weight gain of approx. 6% was noted for the period from test day 1 to test day 16, being approx. 10 percentage points lower compared to the control group. No noteworthy difference was noted for the body weight at autopsy between the females treated with **100 µg BNT162b2/animal** and the females of the control group on test day 17.

Recovery period

No noteworthy changes were noted for the body weight, body weight gain, and body weight at autopsy of the male animals previously treated with **100 µg BNT162b2/animal** in comparison to the control animals at the end of the recovery period. The body weight of the female animals was consistently slightly lower compared to the control group, despite of their older age. The body gain from test day 16 to test day 37 was nearly identical to that of the control group. At the end of the recovery period, there was no noteworthy difference in body weight between the two groups.

Statistically significant differences observed for the body weight between any test item-treated group and the control group as listed in the text table below are not considered to be test item-related but to be coincidental changes.

Text table 4-3: Statistically significant body weight changes considered not test item-related

Body weight changes compared to the control group considered not test item-related (refer to Table 3-1)							
Group	**Test item no.#**	**Dose [µg/animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]
7	4	100	m	1	+16.0	$p \leq 0.01$	A

[redacted]
Test item 4: BNT162b2 - Group 7
[redacted]

m male
f female

A Change is due to the older age of the animals.

Figure 1-1 Body weight of male rats treated once weekly, mean values per group
Up to 3 administrations per animal (TD 1/8/15)

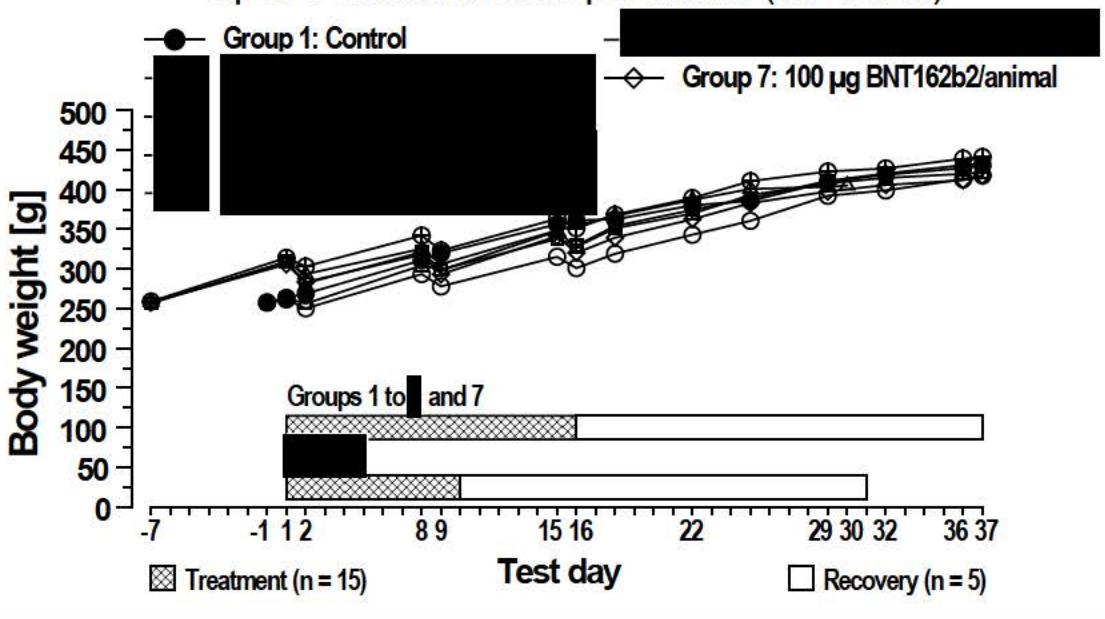


Figure 1-2 Body weight of female rats treated once weekly, mean values per group
Up to 3 administrations per animal (TD 1/8/15)

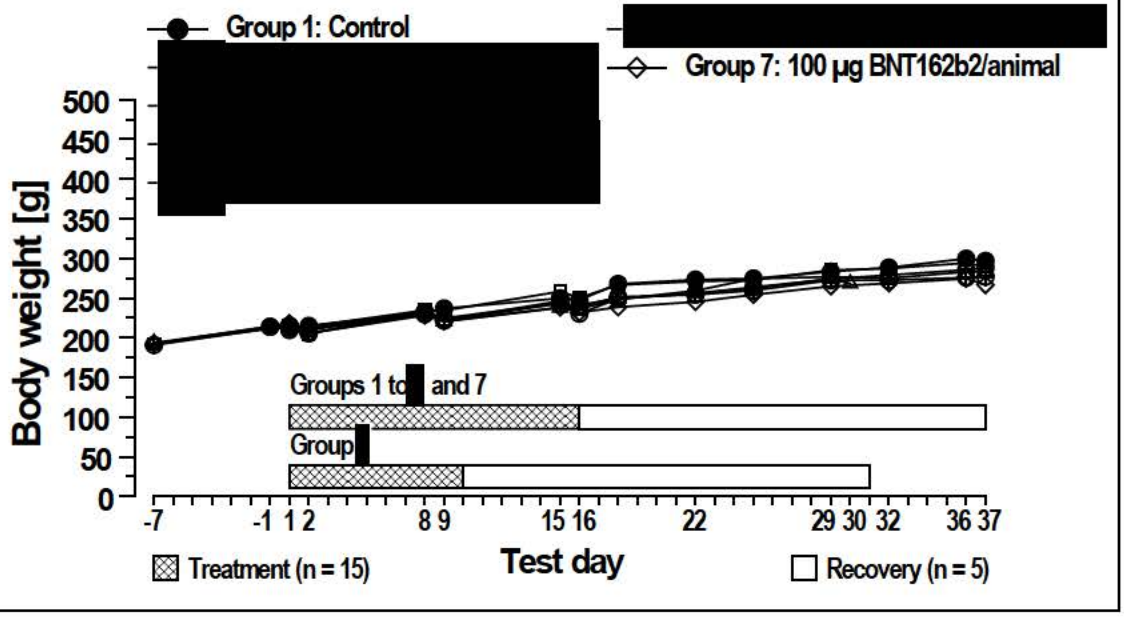


Figure 1-3 Body weight gain of male rats treated once weekly, mean values per group and standard deviation

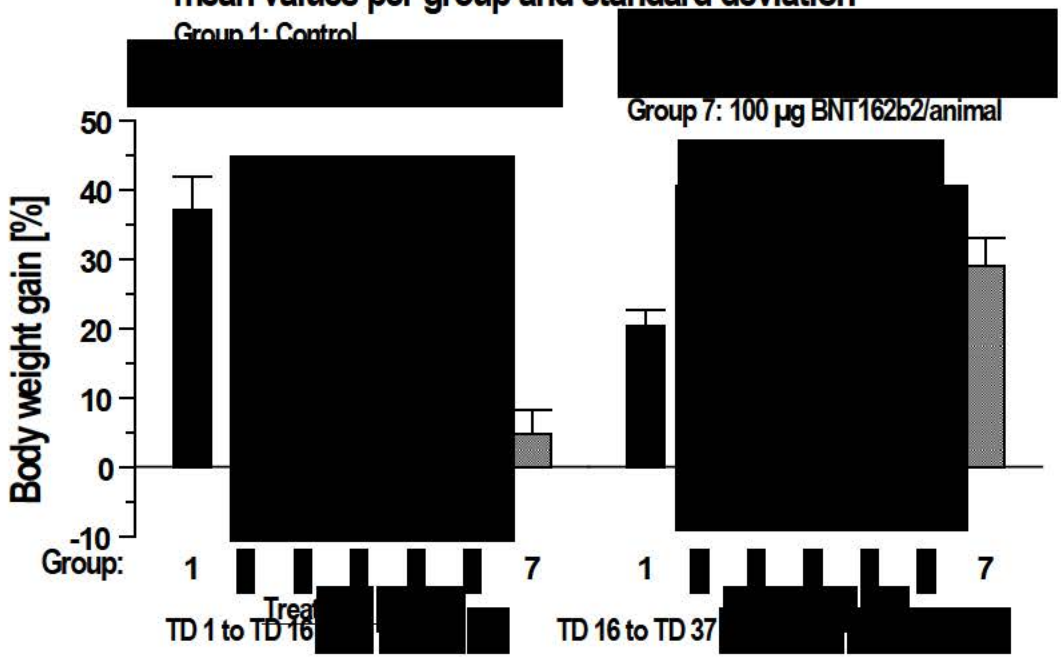


Figure 1-4 Body weight gain of female rats treated once weekly, mean values per group and standard deviation

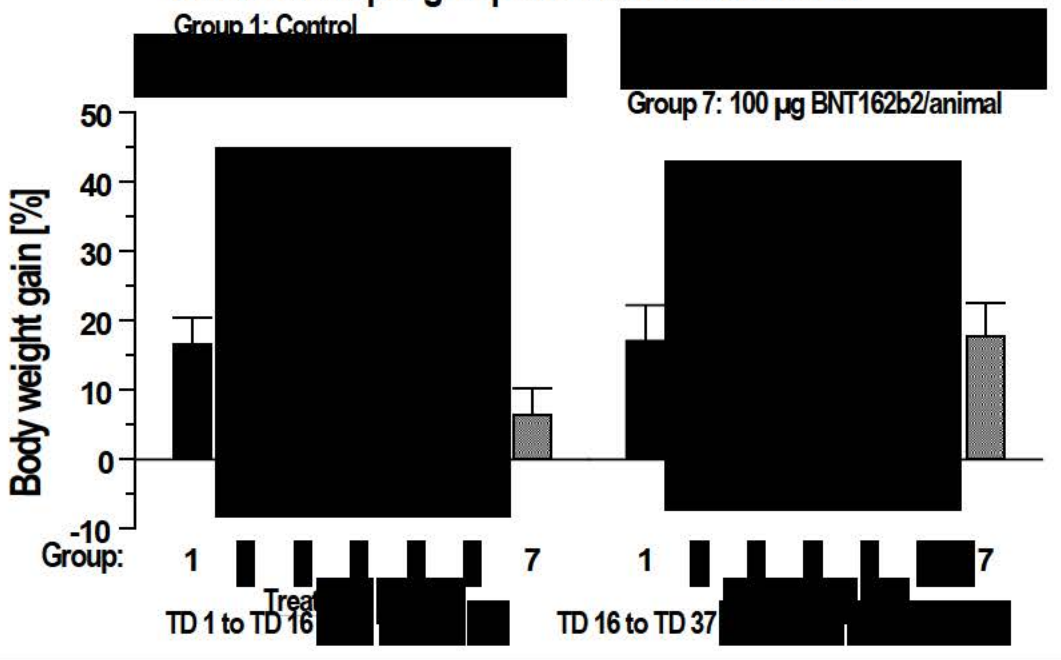


Figure 1-5 Body weight at autopsy, individual data -Males

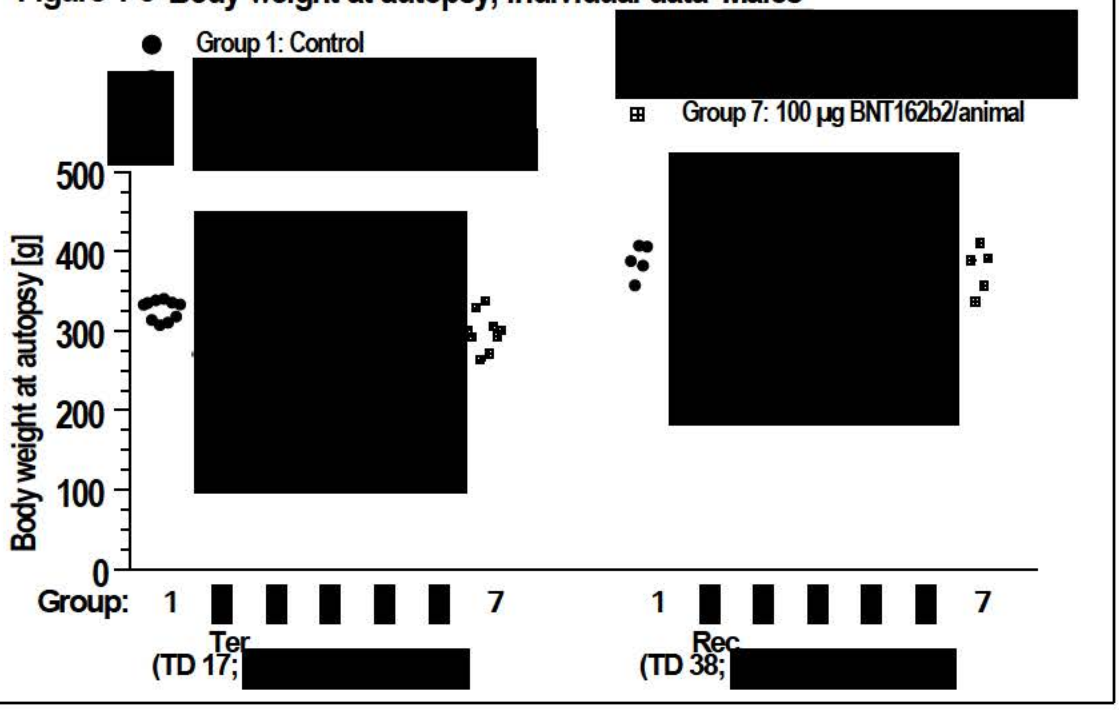
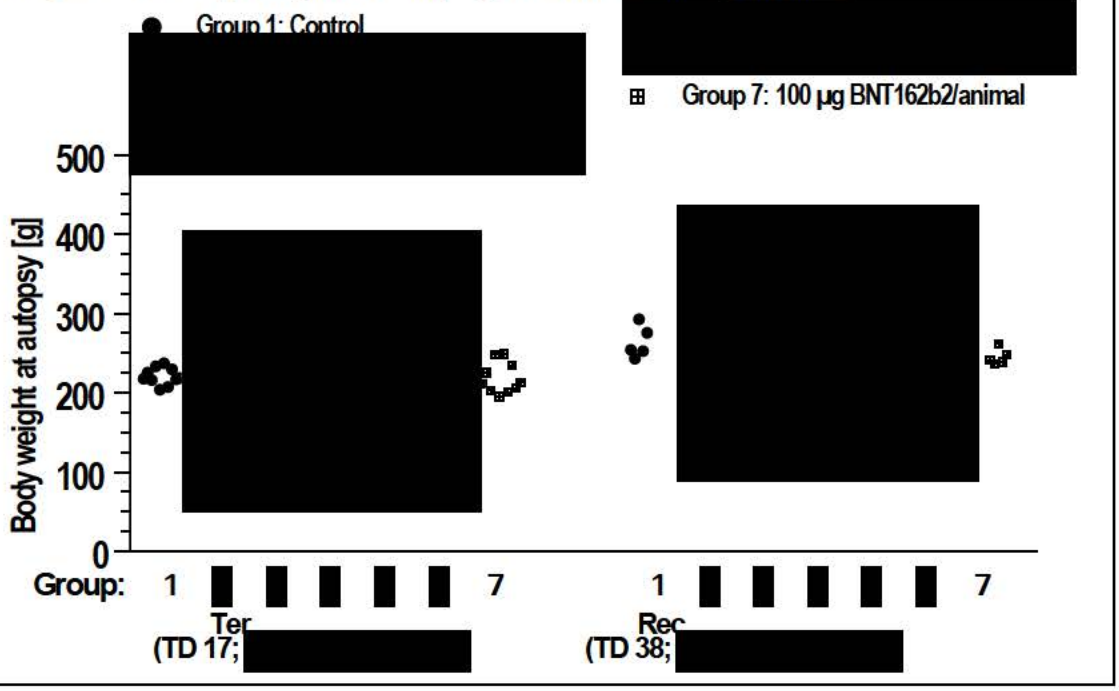


Figure 1-6 Body weight at autopsy, individual data -Females



4.5 Food and drinking water consumption

[redacted]

BNT162b2 - Group 7

Treatment period and recovery period

No test item-related influence was observed on the relative food consumption for the male and female animals following intramuscular treatment with [redacted] **100 µg BNT162b2/animal** (group 7) on test days 1, 8, and 15 (3 administrations), [redacted] in comparison to the control group throughout the treatment and recovery period.

Any differences to the control group are regarded to be within the normal range of biological variation.

The statistically significant differences to the control group's relative food consumption that are not considered to be related to any of the test items are listed in the text table on the following page.

Text table 4-4: Statistically significant changes in relative food consumption considered not test item-related

Statistically significant changes in relative food consumption (refer to Table 4-1) in comparison to the control group considered not test-item-related							
Group	**Test item no.#**	**Dose [µg/animal]**	**Sex**	**Test week**	**Change [%]**	**Statistical significance**	**Reason**
[redacted]							
7	4	100	m	1	-22.3	$p \leq 0.01$	C
				2	-11.1	$p \leq 0.01$	C
				3	-13.4	$p \leq 0.01$	C
			f	1	-13.7	$p \leq 0.01$	C
				4	+13.2	$p \leq 0.01$	B

[redacted]
Test item 4: BNT162b2 - Group 7
[redacted]

m male
f female

A Change is within limits of normal biological variation and without toxicological relevance.
B Change is due to the lower weight of the respective animals.
C Change is due to the higher weight of the respective animals.

Visual appraisal of the drinking water consumption did not reveal any noteworthy differences between any of the test item-treated groups and the control group throughout the treatment and recovery period. The consumption was not quantified.

Mean values per group and individual data of food intake are listed in Table 4-1 (Food Consumption - Summary) and Table 4-2 (Food Consumption - Individual Data).

The mean relative food consumption (in g/kg b.w./day) per group and sex is shown graphically in Figure 2-1 (males) and Figure 2-2 (females) on the following page.

Figure 2-1 **Food consumption of male rats treated once weekly, mean values per group**

Up to 3 administrations per animal (TD 1/8/15)

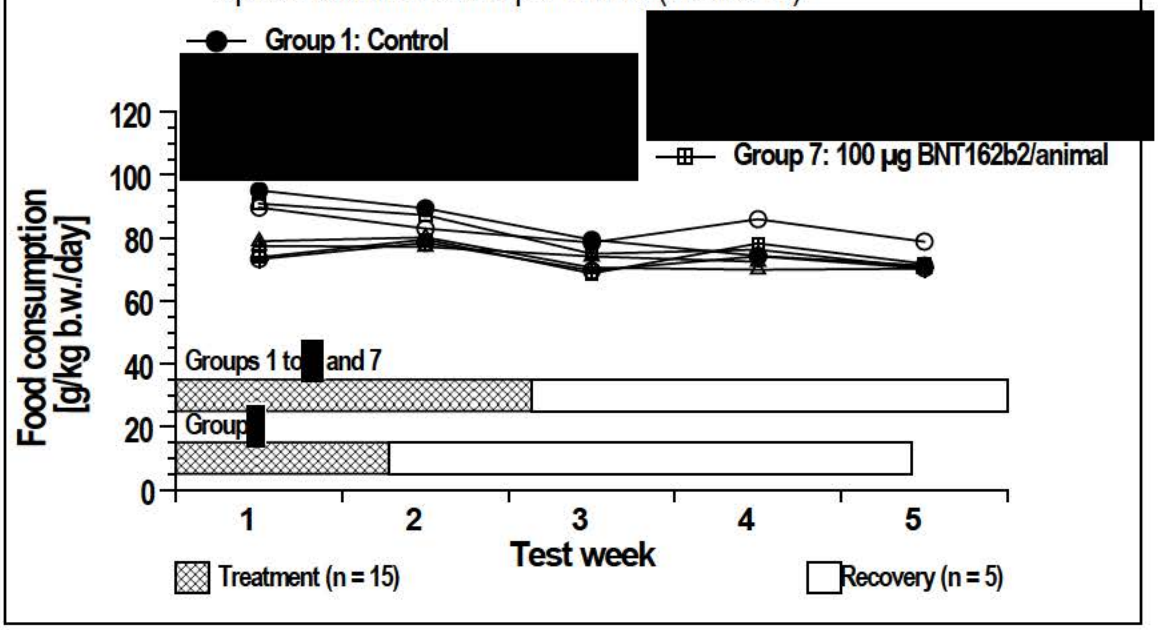
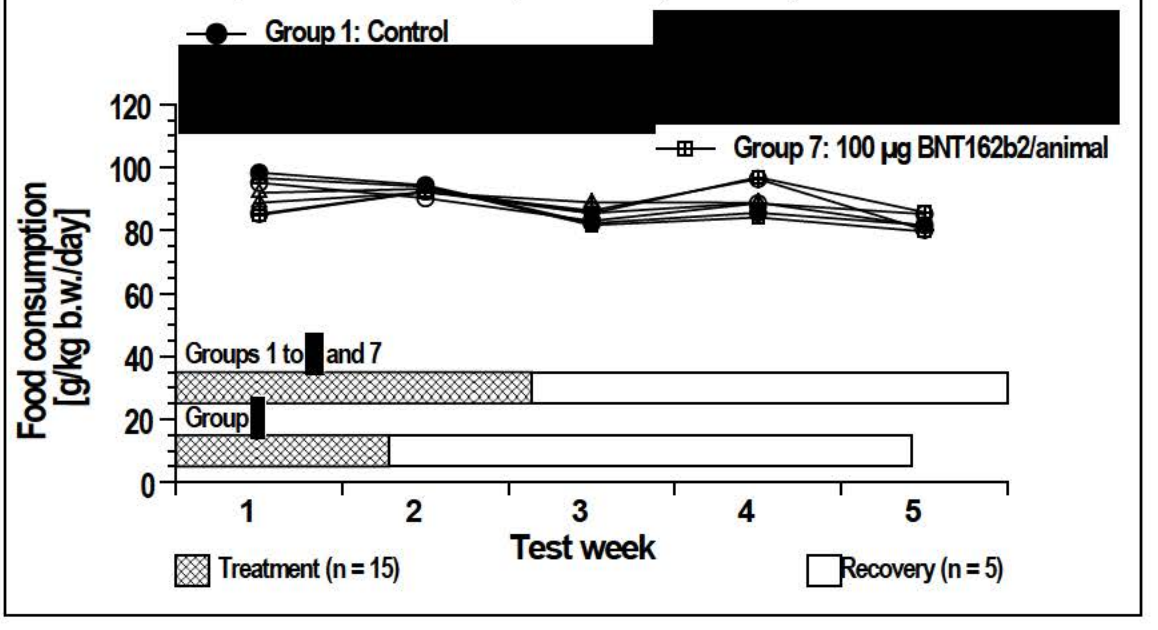


Figure 2-2 **Food consumption of female rats treated once weekly, mean values per group**

Up to 3 administrations per animal (TD 1/8/15)



4.6 Body temperature

[redacted]

[redacted] **BNT162b2 - Group 7**

Treatment and recovery period

Intramuscular treatment with [redacted] **100 µg BNT162b2/animal** (group 7) on test days 1, 8, and 15 (3 administrations), [redacted] led to slightly increased body temperatures at 4 h p.a. and/or 24 h p.a. compared to the control animals (statistically significant at $p \leq 0.01$ or $p \leq 0.05$ in many cases). The effect appeared to be slightly more pronounced in the groups treated with the higher test item dose levels (i.e. groups [redacted] and 7).

During the recovery period, the body temperature remained at a slightly higher level compared to the control group in all previously test item treated groups.

The slight body temperature *decreases* noted for groups [redacted] and 7 in comparison to the control group are considered to be due to the circumstances of the time-shift in study conduct for these groups. The body temperature measurements of these animals were performed on dates different from those for the control and by different staff.

Mean values per group and individual data of body temperature are listed in Table 5-1 (Body Temperature - Summary) and Table 5-2 (Body Temperature - Individual Data).

The mean body temperature per group and sex is shown graphically in Figure 3-1 (males) and Figure 3-2 (females) on the following page.

Figure 3-1 Body temperature of male rats treated once weekly, mean values per group
Up to 3 administrations per animal (TD 1/8/15)

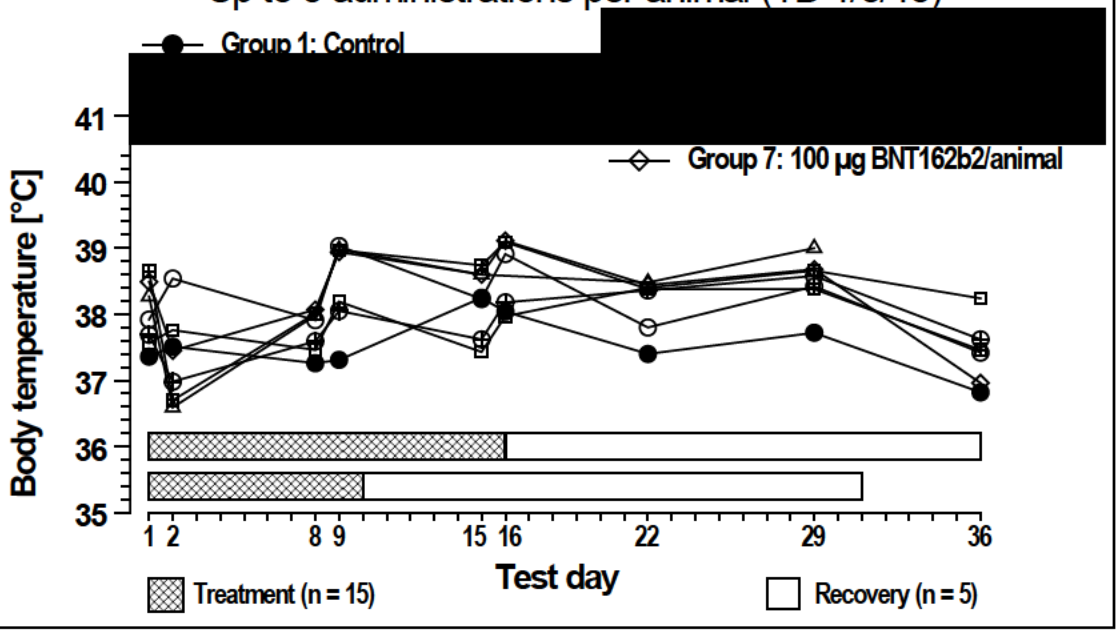
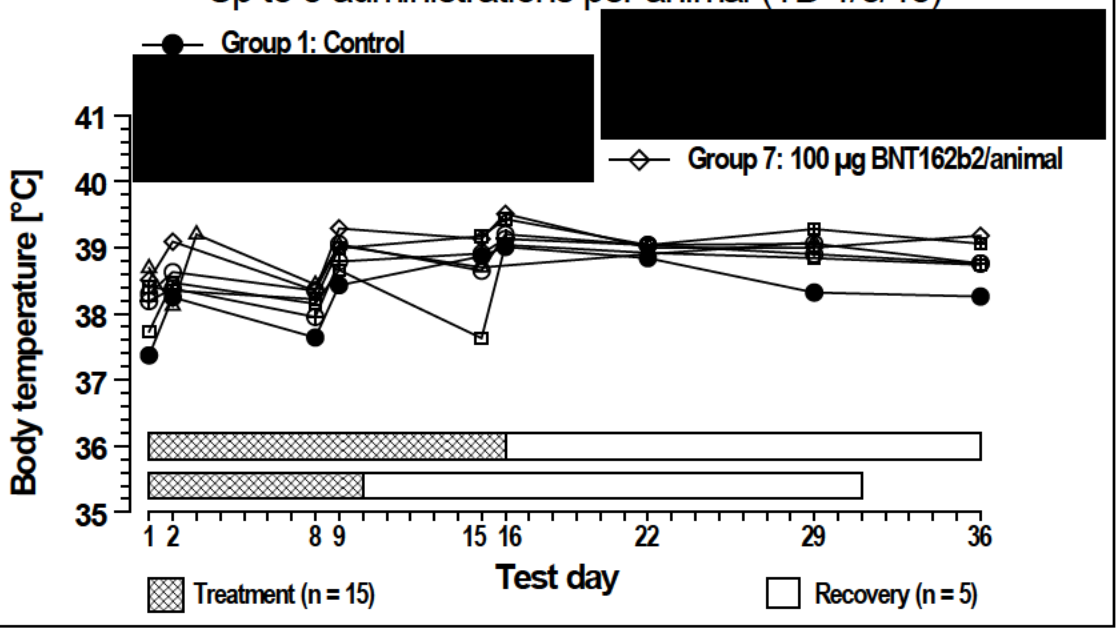


Figure 3-2 Body temperature of female rats treated once weekly, mean values per group
Up to 3 administrations per animal (TD 1/8/15)



4.7 Haematology and coagulation

The most consistent test item-related haematologic changes were dose-related increases in neutrophils and large unstained cells (LUC), which were seen with all test items on test day 17, but were greatest in groups [redacted] and 7 and were greater in females relative to males. Other test item-related changes included decreases in the absolute and relative reticulocyte count (test day 4 only), platelet count, and red cell mass (HGB, HCT and RBC; test day 17 only), and increases in the numbers of leucocytes, monocytes, eosinophils, basophils and/or fibrinogen concentrations. All changes were considered to be related to the primary pharmacodynamic activity of the vaccines, which induce a potent immune response.

Increases in fibrinogen levels and leucocytes (most notably neutrophils and LUC), were consistent with an acute phase response secondary to immune activation and inflammation at the injection sites.

Decreases in numbers of reticulocytes, RBC and platelets were associated with increased bone marrow haematopoiesis, consistent with transient, secondary or peripheral effects. Transient reticulocyte decreases (test day 4 only) were likely secondary to the acute phase response and inflammation. Effects on red cell mass were limited to minimal decreases in RBC, HGB, and HCT on test day 17. Platelet decreases were small in magnitude and not expected to result in bleeding. They were likely secondary to inflammation-related platelet activation and consumption. There were no thrombi evident microscopically.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted] [redacted]

[redacted]		
[redacted]	[redacted]	
	[redacted]	[redacted]
[redacted]		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]		

BNT162b2 - Group 7

Treatment period

Test item-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, eosinophils, monocytes, large unstained cells (LUC), basophils and/or the levels of fibrinogen. All changes fully reversed by the end of the recovery phase.

The test item-related changes noted for the animals treated with **100 µg BNT162b2/animal** (group 7) as given in the text table on the following page.

Text table 4-8: Test item-related changes in haematological and coagulation parameters for the treatment with BNT162b2

Test item-related changes in haematological and coagulation parameters, group 7 compared to the control group in % (refer to Table 6-1)		
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		
Haemoglobin (HGB)	-9.1**	-12.7**
Erythrocytes (RBC)	None	-9.8**
Haematocrit (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).

Recovery period

No noteworthy differences were noted for any haematological or coagulation parameter between the animals previously treated with **100 µg BNT162b2/animal** (group 7) and the control animals at the end of the treatment period. All test item-related changes previously noted during the treatment period had subsided.

No test item-related effects were observed for the the numbers of lymphocytes, the prothrombin time (PT), the activated partial thromboplastin time (aPTT), the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC), the mean platelet (thrombocyte) volume (MPV), the relative volume of thrombocytes / Plateletcrit (PCT), the platelet distribution width (PDW), the red cell distribution width (RDW), and the mean platelet component (MPC) for any of the test items during the treatment period and at the end of the recovery period. All data of the parameters given before are considered to be within the normal range of biological variability.

Statistically significant differences in haematological and coagulation parameters noted in comparison to the control group during the treatment period or at the end of the recovery period that are not considered to be test item-related but to be coincidental are listed in the text table starting below.

Text table 4-9: Statistically significant differences in haematological and coagulation parameters considered not test item-related

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)								
Parameter	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
Haemoglobin content (HGB)	[redacted]							
	7	4	100	m	4	+5.9	p ≤ 0.01	A
Erythrocytes (RBC)								
	7	4	100	m	4	+8.0	p ≤ 0.01	A
Leucocytes (WBC)								
	7	4	100	m	4	+37.0	p ≤ 0.01	A

- Text table continued on the next page -

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Reticulocytes (rel.)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	17	-23.3	p ≤ 0.01	A
Reticulocytes (abs.)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m		-26.3	p ≤ 0.01	A
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] value (HCT)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]	[redacted]	[redacted]
Neutrophils (Neut), abs.	7	4	100	f	4	+126.9	p ≤ 0.01	A
Lymphocytes (Lym), abs.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	4	+36.8	p ≤ 0.01	A
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Basophils (Baso), abs.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	f	4	+65.4	p ≤ 0.05	A
Activated partial thromboplastin time (aPTT)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	17	+14.1	p ≤ 0.05	A
				f	17	+18.1	p ≤ 0.01	A
- Text table continued on the next page -								

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Mean corpuscular volume (MCV)	[redacted]							
	7	4	100	m	4	-5.3	p ≤ 0.01	A
					17	-8.6	p ≤ 0.01	A
				f	17	-4.0	p ≤ 0.05	A
Mean corpuscular haemoglobin (MCH)	[redacted]							
	7	4	100	m	17	-6.0	p ≤ 0.01	A
Mean corpuscular haemoglobin concentration (MCHC)	[redacted]							
	7	4	100	m	4	+3.7	p ≤ 0.01	A
					17	+3.2	p ≤ 0.01	A
				f	4	+3.0	p ≤ 0.01	A

- Text table continued on the next page -

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Mean platelet (thrombocyte) volume (MPV)	[redacted]							
	7	4	100	m	17	-14.2	p ≤ 0.01	A
				f	17	-11.6	p ≤ 0.01	A
Relative volume of thrombocytes / Plateletcrit (PCT)	[redacted]							
	7	4	100	m	17	-38.7	p ≤ 0.01	A, B
				f	17	-43.2	p ≤ 0.01	A, B
Platelet distribution width (PDW)	[redacted]							
	7	4	100	m	4	+24.7	p ≤ 0.01	A
					17	+45.8	p ≤ 0.01	A, B
				f	17	+42.4	p ≤ 0.01	A, B

- Text table continued on the next page -

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Red cell distribution width (RDW)	[redacted]							
	7	4	100	m	17	-12.0	$p \leq 0.01$	A
					38	+15.3	$p \leq 0.01$	A, B
				f	17	-6.2	$p \leq 0.05$	A
					38	+14.8	$p \leq 0.01$	A, B
Mean platelet component (MPC)	[redacted]							
- Text table continued on the next page -								

Statistically significant differences in haematological and coagulation parameters in comparison to the control group considered not test item-related (refer to Table 6-1)								
Parameter	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
- Text table continued from previous page -								
Mean platelet component (MPC) - *continued*	[redacted]							
	7	4	100	m	4	+15.8	p ≤ 0.01	A
					17	+18.7	p ≤ 0.01	A, B
				f	4	+14.8	p ≤ 0.01	A
					17	+18.3	p ≤ 0.01	A, B
					38	+14.4	p ≤ 0.01	A

[redacted]
Test item 4: BNT162b2 - Group 7
[redacted]

m male
f female

abs. absolute count
rel. relative count

A Change is within the limits of normal biological variation (with regard to the range covered by the control group) and without toxicological relevance.
B Change is due to the relative high or low value noted for the control group.

Group mean values of haematological and coagulation parameters are presented in Table 6-1 (Haematological Parameters - Summary), individual data are listed in Table 6-2 (Haematological Parameters - Individual Data).

4.8 Clinical chemistry

[redacted]

[redacted] **BNT162b2 - Group 7**

Treatment period

An elevated plasma activity of gamma-glutamyltransferase (gamma-GT) was noted for all test item-treated groups in comparison to the control group as given in the text table below. There were no macroscopic or microscopic findings (see Section 4.14 and Section 4.16) consistent with cholestasis or hepatobiliary injury to explain the increased gamma-GT.

Text table 4-10: Test item-related changes in plasma activity of gamma-glutamyltransferase

Test item-related changes in plasma activity of gamma-glutamyltransferase (gamma-GT) compared to the control group in % (refer to Table 7-1)		
[redacted]		
[redacted]		
Test day	Group 7: 100 µg BNT162b2/animal	
	Males	Females
4	+242.1**	+355.7**
10	[redacted]	
17	+198.1**	+317.4**

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

↑ Increase relative to study control range, but % difference not quantifiable due to lacking concurrent controls.

The range of individual data noted for the gamma-GT activity in several test groups slightly exceeded the range of [redacted] historical data as summarised in the text table on the following page.

Text table 4-11: Comparison of gamma-glutamyltransferase activity observed in this study to [redacted] historical data

Enzyme activity of gamma-glutamyltransferase (gamma-GT) [U/mL plasma]					
Sex	Group	Data observed in this study[#1] Mean (Range of individual data)			[redacted] historical Data[#2] Mean (Range of individual data)
		TD 4	TD 10[#3]/17	TD 31[#3]/38	
Males	1	0.95 (0.1 - 2.4)	1.62 (0.7 - 2.9)	2.60 (1.6 - 3.6)	2.28 (0.4 - 5.1)
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	7	3.25 (1.3 - 4.9)	4.83 (3.6 - 6.5)	1.82 (0.5 - 3.2)	
Females	1	0.88 (0.1 - 1.9)	1.21 (0.6 - 2.4)	2.48 (1.2 - 3.0)	2.43 (0.2 - 4.8)
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	[redacted]	[redacted]			
	7	4.01 (3.2 - 5.9)	5.05 (4.5 - 6.4)	2.44 (1.8 - 3.5)	

[#1] Age of animals:
Groups 1, [redacted]: 57 days on test day 4, 70 days on test day 17, 91 days on test day 38.
Groups [redacted] and 7: 63 days on test day 4, 76 days on test day 17, 97 days on test day 38.
Group [redacted]: 63 days on test day 4, 69 days on test day 10, 90 days on test day 31.

[#2] Obtained from 3 studies conducted at [redacted] from 2016 to 2018 (in total 45 control group animals per sex, age at examination: 30 to 92 days, treated only with physiological saline or vehicle). The data were not audited by [redacted] QAU.

[#3] [redacted]

TD Test day

A graphical presentation of the gamma-glutamyltransferase activity is given in Figure 4-1 (males) and Figure 4-2 (females) on the following page.

Figure 4-1 Gamma-glutamyltransferase plasma activity in male rats mean values per group and standard deviation

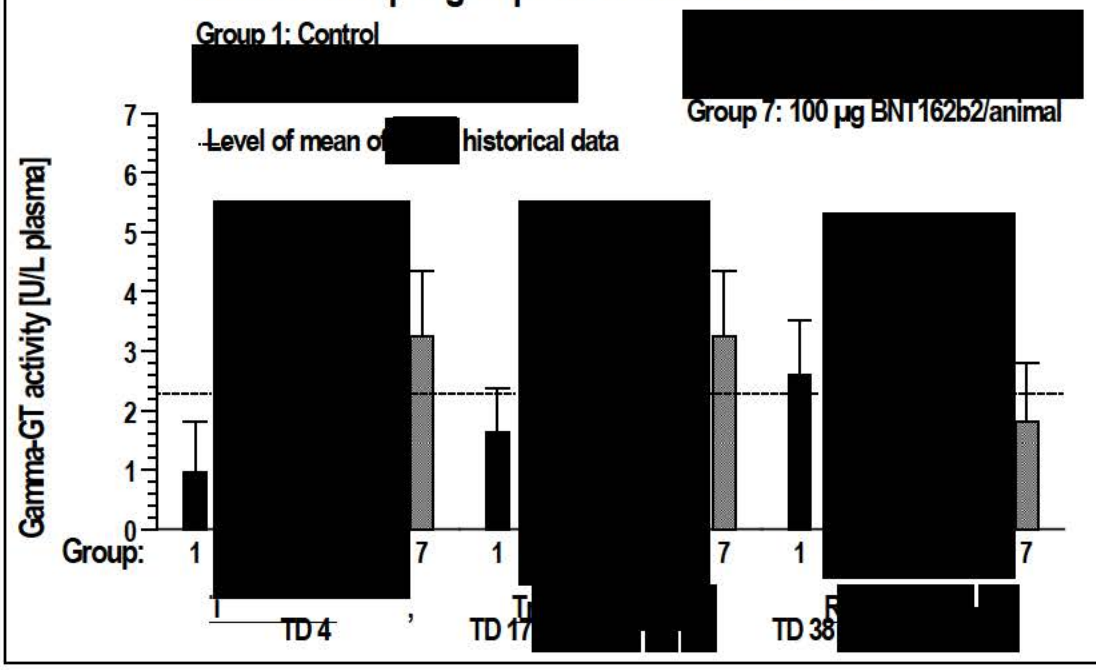
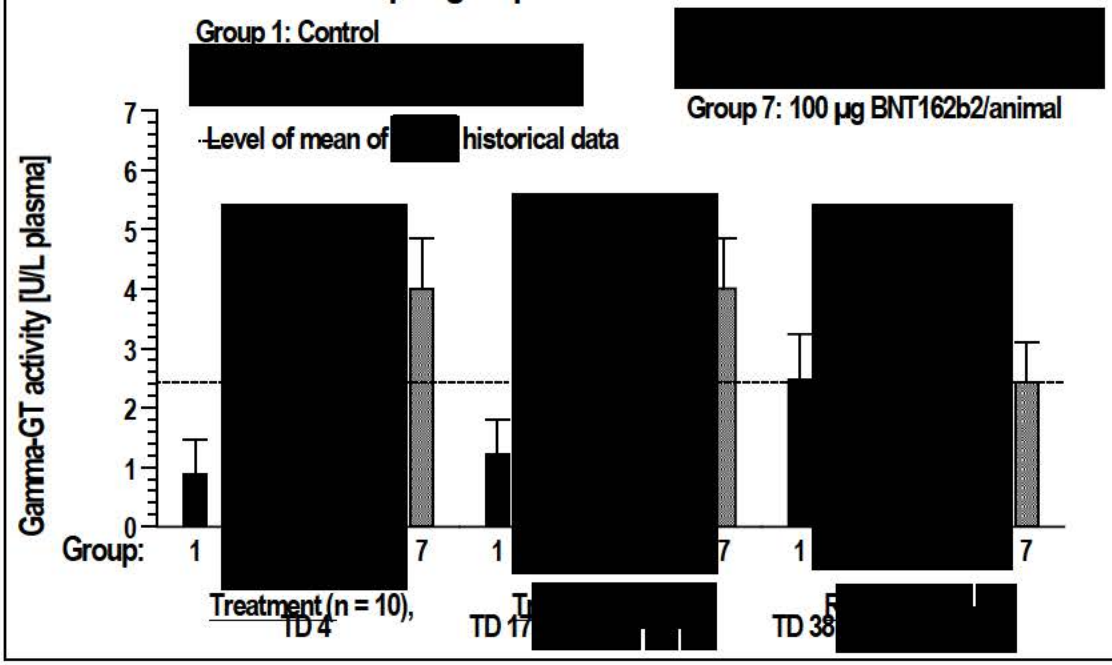


Figure 4-2 Gamma-glutamyltransferase plasma activity in female rats mean values per group and standard deviation



The elevated plasma activity of gamma-glutamyltransferase is considered to be related to the test item administration, but the cause is unclear.

Further, a decrease in albumin plasma levels and an increase in globulin plasma levels, resulting in an altered albumin/globulin ratio, were observed in all test item treated groups. The 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 statistically significant changes noted in albumin and globulin levels and the alb./glob. ratio are listed in the text table below.

Text table 4-12: Statistically significant differences in albumin and globulin levels and the albumin/globulin ratio

Statistically significant differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group (refer to Table 7-1)						
Parameter	**Group**	**Test item**	**Dose [µg/animal]**	**Sex**	**Test day**	**Change [%]**
Albumin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
	7	BNT162b2	100	m	4	-9.1**
					17	-5.9**
				f	4	-12.6**
					17	-11.0**
Globulin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]

- Text table continued on the next page -

Statistically significant differences in albumin and globulin levels and the albumin/ globulin ratio compared to the control group (refer to Table 7-1)

Parameter	Group	Test item	Dose [µg/animal]	Sex	Test day	Change [%]
- Text table continued from previous page -						
Globulin *- continued*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	BNT162b2	100	m	4	+7.3*
					17	+23.1**
				f	17	+17.7**
Albumin/Globulin Ratio	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]
	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).

No other test item-related influence was observed on any of the biochemical parameters for the animals treated with [redacted] **100 µg BNT162b2/animal** (group 7) on test days 1, 8, and 15, [redacted] compared to the control animals during the treatment period (test day 4, [redacted] test day 17 for groups 1 to [redacted] and 7) and at the end of the recovery period.

Recovery period

The elevated plasma activity of gamma-glutamyltransferase noted during the treatment period had subsided in all test item-treated groups at the end of the recovery period [redacted] test day 38 for all other groups) and was in a range comparable to that of the control group.

No test item-related effects were noted on the plasma levels of total bilirubin, total cholesterol, creatinine, glucose, phosphate, total protein, urea (in blood), triglycerides, calcium, chloride, potassium, and sodium. No test item related influence was noted on the plasma enzyme activities of alanine aminotransferase (ALAT), alkaline phosphatase (aP), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), and creatine kinase (CK). All data of the parameters given above are considered to be within the range of normal biological variability throughout the treatment and recovery period.

Statistically significant differences (at $p \leq 0.01$ or $p \leq 0.05$) were noted for several clinical chemistry parameters between the various test item-treated groups and the control group. The majority of the changes noted were of only marginal degree and occurred in groups [redacted] and 7, for the animals of which the study conduct started at a later time (see Section 2.7). These animals were 6 days older than the control animals at the time of examination. Blood withdrawal and plasma analysis were performed on dates different from those for the control. An influence of this time offset on the analyses' results cannot be completely excluded.

Statistically significant differences in clinical chemistry parameters in comparison to the control group during the treatment period or at the end of the recovery period that are not considered to be test item-related but to be coincidental are listed in the text table on the following page.

Text table 4-13: Statistically significant differences in clinical chemistry parameters considered not test item-related

Statistically significant differences in clinical chemistry parameters in comparison to the control group considered not test item-related (refer to Table 7-1)								
Parameter	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
[redacted]								
[redacted]								
Bilirubin								
	7	4	100	m	4	-25.1	p ≤ 0.01	A
					17	+42.9	p ≤ 0.01	A
				f	4	-22.2	p ≤ 0.01	A
					17	+30.7	p ≤ 0.01	A
Cholesterol								
	7	4	100	m	4	-25.4	p ≤ 0.01	A
					17	-31.9	p ≤ 0.01	A
				f	17	-26.0	p ≤ 0.05	A
Creatinine								

- Text table continued on the next page -

Statistically significant differences in clinical chemistry parameters in comparison to the control group considered not test item-related (refer to Table 7-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Creatinine *- continued*	7	4	100	m	4	+10.1	$p \leq 0.05$	A
					17	+12.0	$p \leq 0.01$	A
				f	17	+7.3	$p \leq 0.05$	A
Glucose	[redacted]							
	7	4	100	m	4	-26.1	$p \leq 0.01$	A
				f	4	-27.8	$p \leq 0.01$	A
[redacted]	[redacted]							
Protein (total)	[redacted]							
	7	4	100	m	17	+7.8	$p \leq 0.01$	A
Triglycerides	[redacted]							
- Text table continued on the next page -								

Statistically significant differences in clinical chemistry parameters in comparison to the control group considered not test item-related (refer to Table 7-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Triglycerides *- continued*	[redacted]							
	7	4	100	m	4	-73.5	p ≤ 0.01	A
				f	4	-71.7	p ≤ 0.01	A
Urea (in blood)	[redacted]							
	7	4	100	m	17	+35.4	p ≤ 0.01	A
Calcium	[redacted]							
	7	4	100	m	4	-6.8	p ≤ 0.01	A
Chloride	[redacted]							
	7	4	100	m	4	+2.1	p ≤ 0.01	A
Potassium	[redacted]							
	7	4	100	m	4	-8.0	p ≤ 0.05	A
				f	17	+10.1	p ≤ 0.05	A
Sodium	[redacted]							
	7	4	100	m	4	+1.6	p ≤ 0.01	A
					17	-1.5	p ≤ 0.01	A

- Text table continued on the next page -

Statistically significant differences in clinical chemistry parameters in comparison to the control group considered not test item-related (refer to Table 7-1)

Parameter	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -								
Alanine amino-transferase (ALAT)	[redacted]							
	7	4	100	m	4	-32.8	$p \leq 0.01$	A
				f	4	-36.5	$p \leq 0.01$	A
Alkaline phosphatase (aP)	[redacted]							
	7	4	100	m	4	-22.3	$p \leq 0.01$	A
				f	17	+142.2	$p \leq 0.01$	A
Aspartate amino-transferase (ASAT)	[redacted]							
	7	4	100	m	4	+19.4	$p \leq 0.01$	A
				f	4	+29.7	$p \leq 0.01$	A
					17	+24.3	$p \leq 0.05$	A

- Text table continued on the next page -

Statistically significant differences in clinical chemistry parameters in comparison to the control group considered not test item-related (refer to Table 7-1)								
Parameter	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
- Text table continued from previous page -								
Lactate dehydro-genase (LDH)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	4	+54.1	$p \leq 0.01$	A
				f	4	+54.8	$p \leq 0.05$	A

[redacted]
Test item 4: BNT162b2 - Group 7
[redacted]

m male
f female

A Change is within the limits of normal biological variation (with regard to the range covered by the control group) and without toxicological relevance.
B Change is due to the relative high or low value noted for the control group.

Group mean values of biochemical parameters are presented in Table 7-1 (Biochemical Parameters - Summary), individual data are listed in Table 7-2 (Biochemical Parameters - Individual Data).

4.9 Acute phase proteins

[redacted]

[redacted] BNT162b2 - Group 7

Treatment period

Elevated serum levels of the acute phase proteins alpha1-acid glycoprotein and alpha2 macroglobulin were noted for all test item-treated groups in comparison to the control group on test day 4 and test day 10 or 17 as given in the text tables following on the following page.

Text table 4-14: Test item-related changes in serum levels of alpha1-acid glycoprotein

Test item-related changes in serum levels of alpha1-acid glycoprotein compared to the control group (refer to Table 8-1), expressed as fold changes (×)		
[redacted]		
Test day	Group 7: 100 µg BNT162b2/animal	
	Males	Females
4	6.9 × **	5.6 × **
10		
17	20.7 × **	15.9 × **

** Statistically significant at p ≤ 0.01 (based on the numerical data, not on the fold change).

↑ Increase relative to study control range, but % difference not quantifiable due to lacking concurrent controls.

Text table 4-15: Test item-related changes in serum levels of alpha2 macroglobulin

Test item-related changes in serum levels of alpha2 macroglobulin compared to the control group (refer to Table 8-1), expressed as fold changes (×)		
Test day		
[redacted]		
Test day	Group 7: 100 µg BNT162b2/animal	
	Males	Females
4	54.3 × **	75.3 × **
10		
17	216.9 × **	120.7 × **

** Statistically significant at p ≤ 0.01 (based on the numerical data, not on the fold change)

↑ Increase relative to study control range, but % difference not quantifiable due to lacking concurrent controls.

All changes noted for the acute phase proteins are considered to be related to the primary pharmacodynamic activity of the intramuscularly delivered vaccines, which induce a local pro-inflammatory environment within the injected muscle and thereby promote a potent immune response.

Recovery period

The elevated serum levels of alpha1-acid glycoprotein and alpha2 macroglobulin noted during the treatment period had subsided in all previously test item-treated groups at the end of the recovery period [redacted] test day 38 for all other groups). The serum levels of both acute phase proteins were in a range comparable to that of the control group in all previously test item-treated groups.

Statistically significant differences in serum levels of acute phase proteins in comparison to the control group noted during the treatment period or at the end of the recovery period that are not considered to be test item-related but to be coincidental are listed in the text table below.

Text table 4-16: Statistically significant differences in serum levels of acute phase proteins considered not test item-related

Statistically significant differences in serum levels of acute phase proteins in comparison to the control group considered not test item-related (refer to Table 8-1)								
Acute phase proteins	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
Alpha1-acid glycoprotein	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	38	-47.8	$p \leq 0.01$	A
Alpha2 macroglobulin	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	38	+63.7	$p \leq 0.05$	A

[redacted]
Test item 4: BNT162b2 - Group 7

m male
f female

A Change is due to the relative high or low value noted for the control group.

Mean values per group and individual data of acute phase proteins are listed in Table 8-1 (Acute Phase Protein Levels - Summary) and Table 8-2 (Acute Phase Protein Levels - Individual Data).

The mean acute phase protein levels per group and sex are shown graphically in Figure 5-1 (Alpha1-acid glycoprotein, males), Figure 5-2 (Alpha1-acid glycoprotein, females), Figure 6-1 (Alpha2 macroglobulin, males), and Figure 6-2 (Alpha2 macroglobulin, females) on the following pages.

Figure 5-1 Alpha1-acid glycoprotein levels in serum of male rats mean values per group and standard deviation

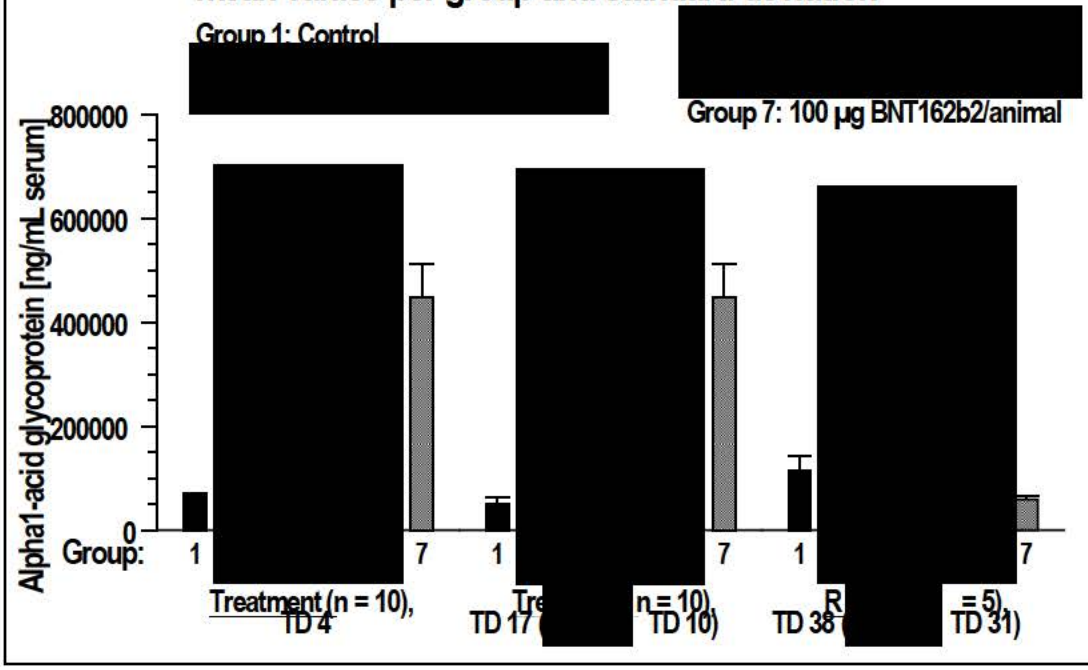


Figure 5-2 Alpha1-acid glycoprotein levels in serum of female rats mean values per group and standard deviation

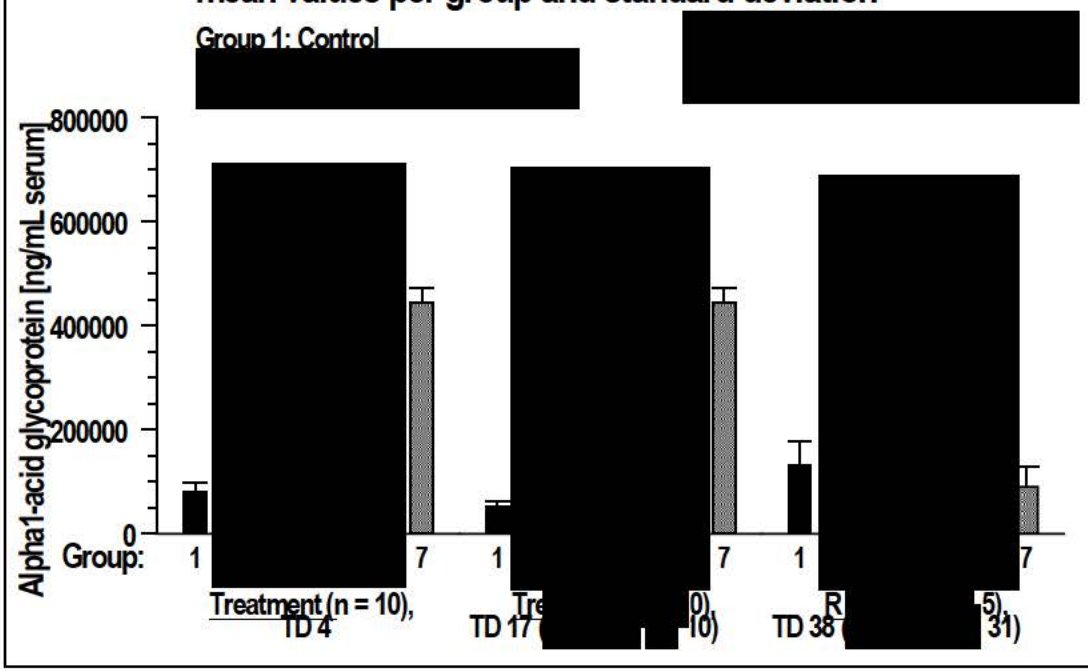


Figure 6-1 Alpha2 macroglobulin levels in serum of male rats mean values per group and standard deviation

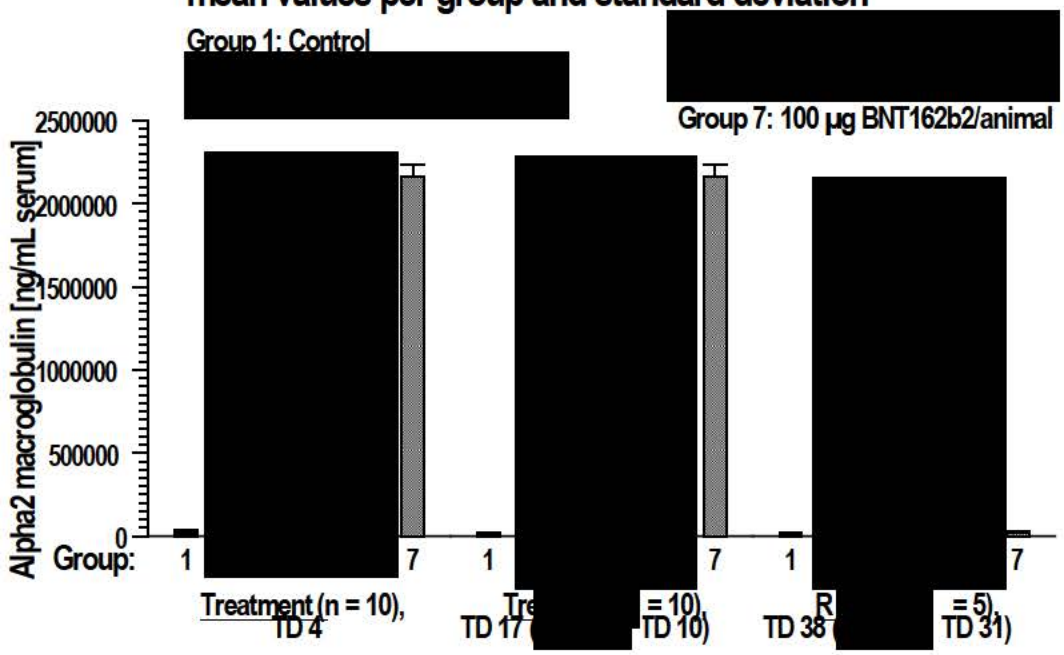
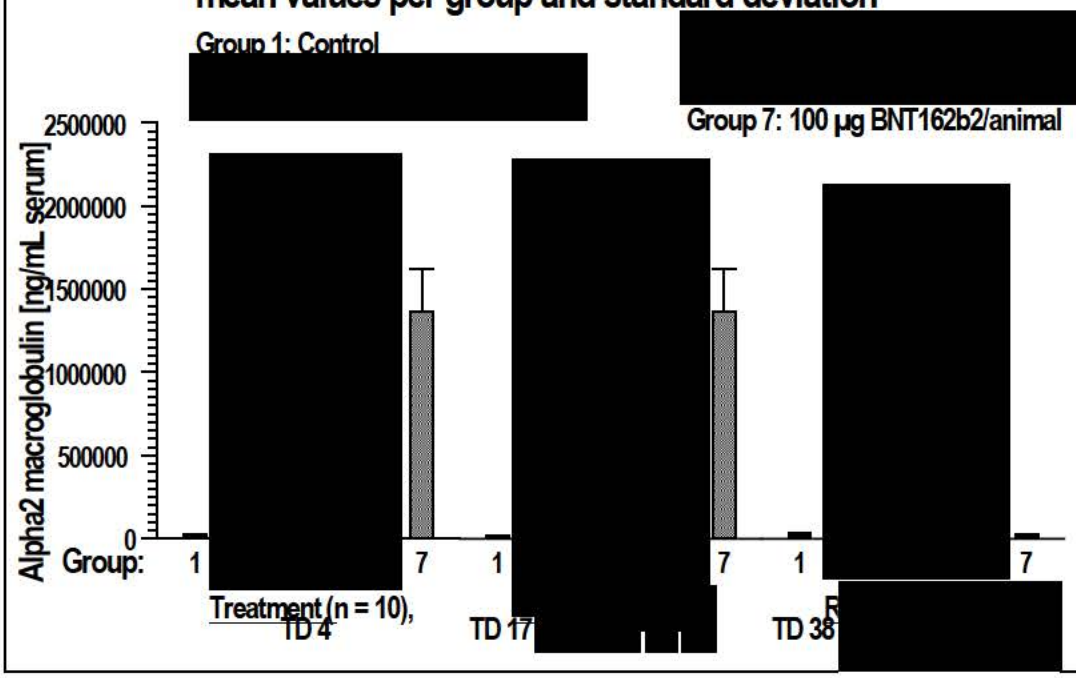


Figure 6-2 Alpha2 macroglobulin levels in serum of female rats mean values per group and standard deviation



4.10 Cytokines

[redacted]

[redacted] BNT162b2 - Group 7

Treatment period and recovery

Elevated serum levels of the cytokines IFN-gamma, TNF-alpha, IL-1beta, IL-6, and IL-10 were noted in all study groups, including the control, compared to the respective predose value as of 6 h p.a. on test day 1. There were no general differences between the test item-treated groups and the control group and among the various test item-treated groups.

A large variability of data was observed using only 3 or 5 animals per group and sex. Therefore, all data obtained are considered to be within the normal range of biological variation. Any differences between the test item-treated animals and the control group are considered as coincidental changes.

Statistically significant differences in cytokine serum levels in comparison to the control group during the treatment period or at the end of the recovery period that are not considered to be test item-related but to be coincidental changes are listed in the text table below.

Text table 4-17: Statistically significant differences in cytokine serum levels considered not test item-related

Statistically significant differences in cytokine serum levels in comparison to the control group considered not test item-related (refer to Table 9-1)								
Cytokine	Group	Test item no.#	Dose [µg/ animal]	Sex	Test day (time point)	Change [%]	Statistical significance	Reason
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

- Text table continued on the next page -

Statistically significant differences in cytokine serum levels in comparison to the control group considered not test item-related (refer to Table 9-1)								
Cytokine	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day (time point)**	**Change [%]**	**Statistical significance**	**Reason**
- Text table continued from previous page -								
IL-1beta	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	7	4	100	m	8 (Predose)	-88.6	p ≤ 0.05	B, C
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
					[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
				[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

Test item 4: BNT162b2 - Group 7

[redacted]

m male
f female

A Change observed predose to start of administration.
B Change is within the limits of normal biological variation (with regard to the range covered by the control group) and without toxicological relevance.
C Change is due to the relative high or low value noted for the control group.
D Change due to mean at level of LLOQ (all individual data are equal to LLOQ).

Mean values per group and individual data of cytokine levels are listed in Table 9-1 (Cytokine Levels - Summary) and Table 9-2 (Cytokine Levels - Individual Data).

The mean cytokine levels per group and sex are shown graphically in Figure 7-1 (IFN-gamma, males), Figure 7-2 (IFN-gamma, females), Figure 8-1 (TNF-alpha, males), Figure 8-2 (TNF-alpha, females), Figure 9-1 (IL-1beta, males), Figure 9-2 (IL-1beta, females), Figure 10-1 (IL-6, males), Figure 10-2 (IL-6, females), Figure 11-1 (IL-10, males), and Figure 11-2 (IL-10, females) on the following pages.

Figure 7-1 IFN-gamma levels in serum of male rats mean values per group and standard deviation

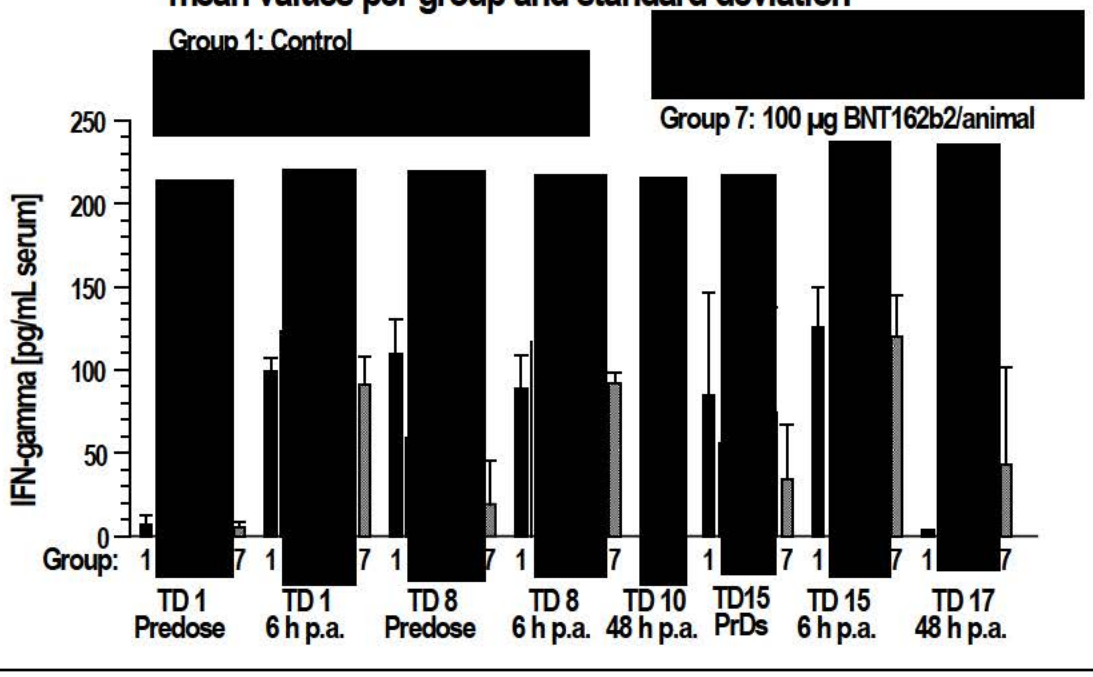


Figure 7-2 IFN-gamma levels in serum of female rats mean values per group and standard deviation

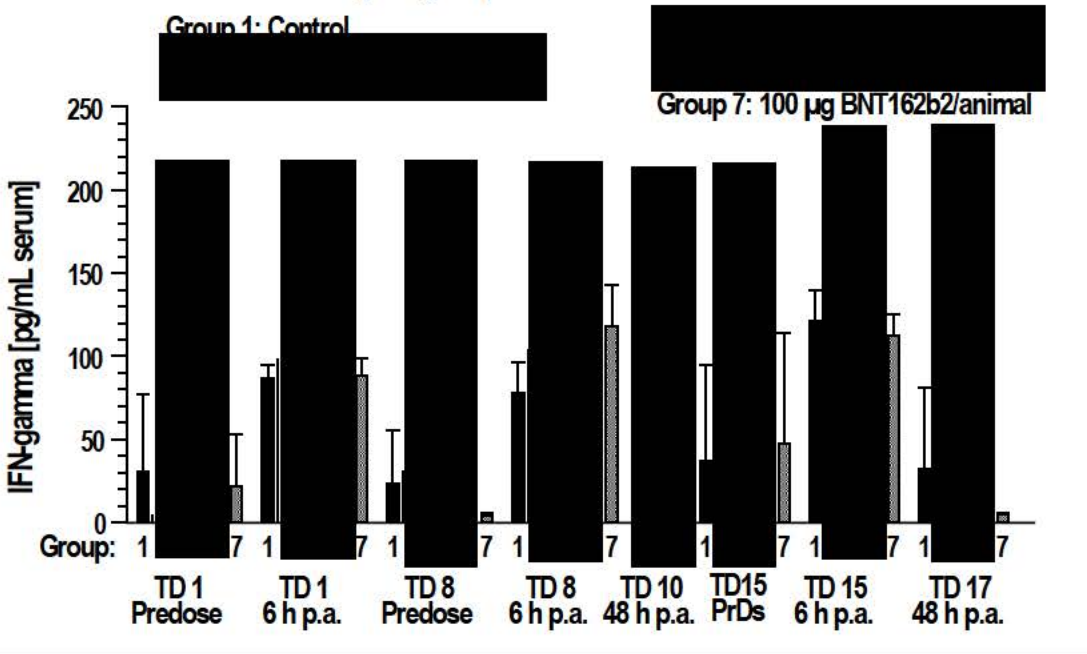


Figure 8-1 TNF-alpha levels in serum of male rats mean values per group and standard deviation

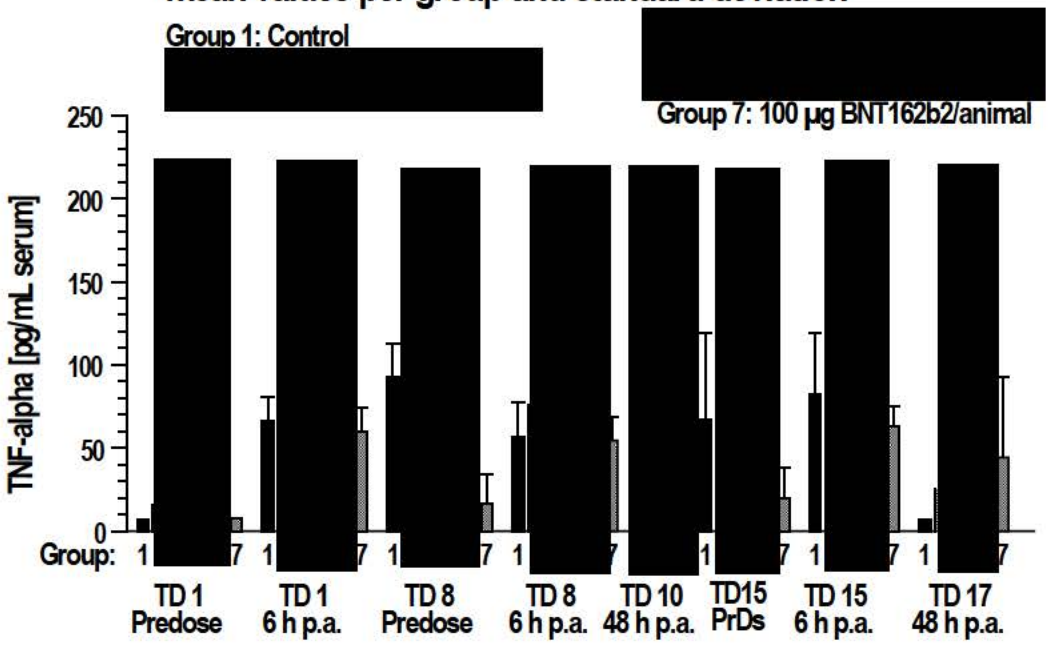


Figure 8-2 TNF-alpha levels in serum of female rats mean values per group and standard deviation

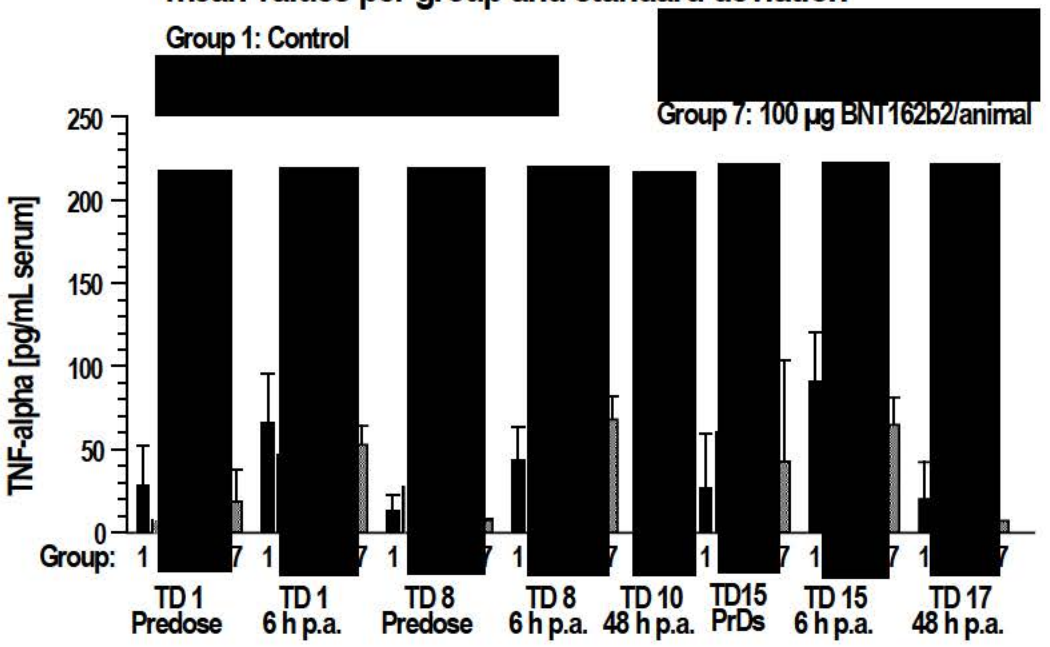


Figure 9-1 IL-1beta levels in serum of male rats mean values per group and standard deviation

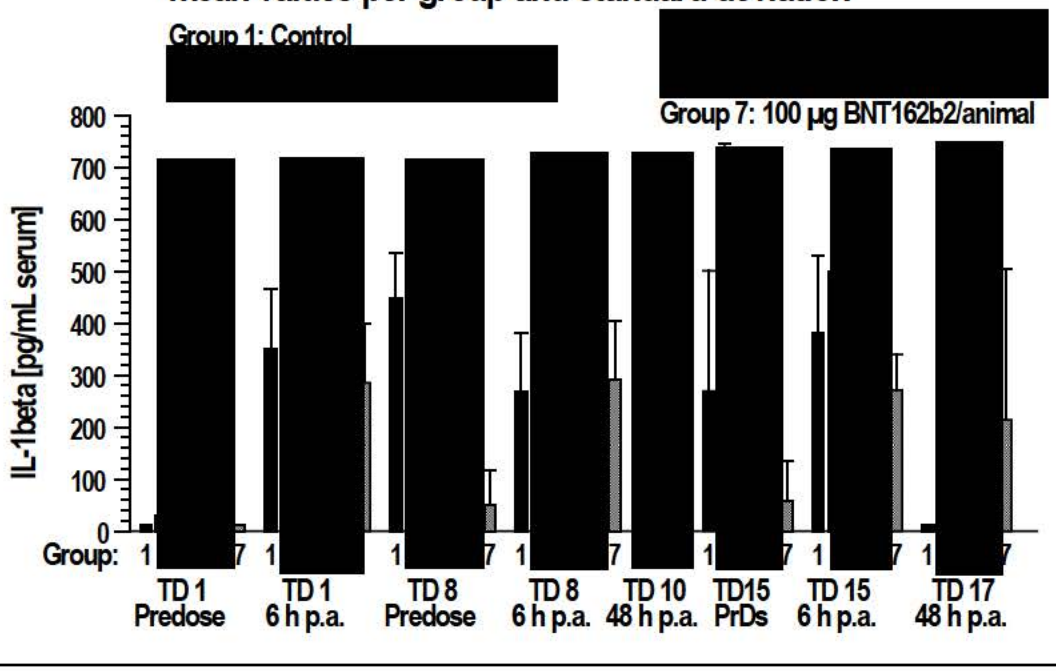


Figure 9-2 IL-1beta levels in serum of female rats mean values per group and standard deviation

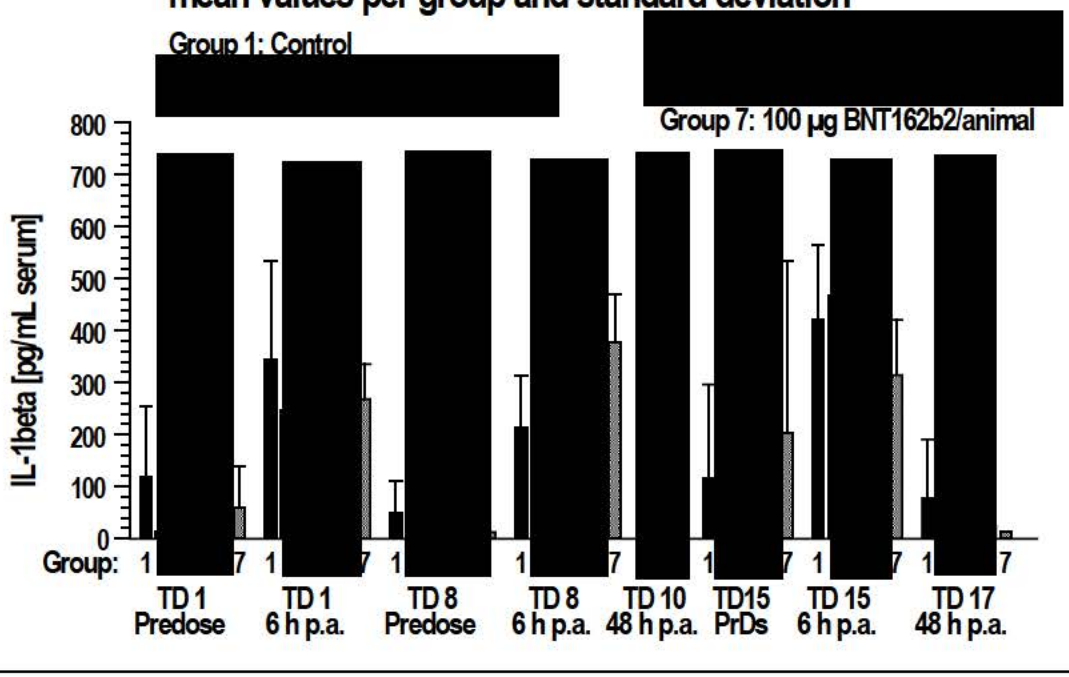


**Figure 10-1 IL-6 levels in serum of male rats
mean values per group and standard deviation**

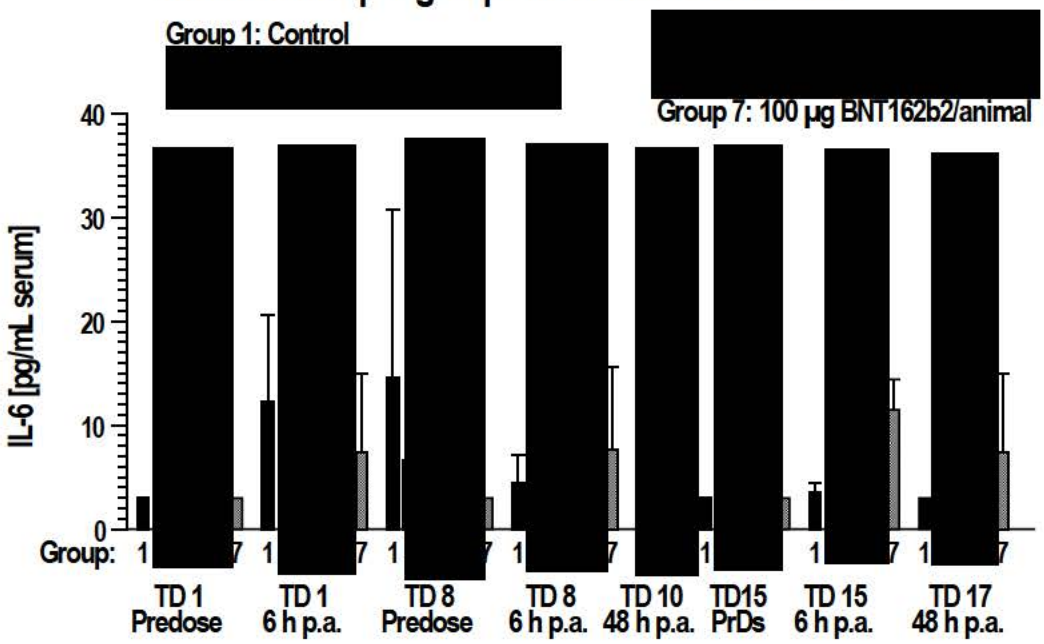


**Figure 10-2 IL-6 levels in serum of female rats
mean values per group and standard deviation**

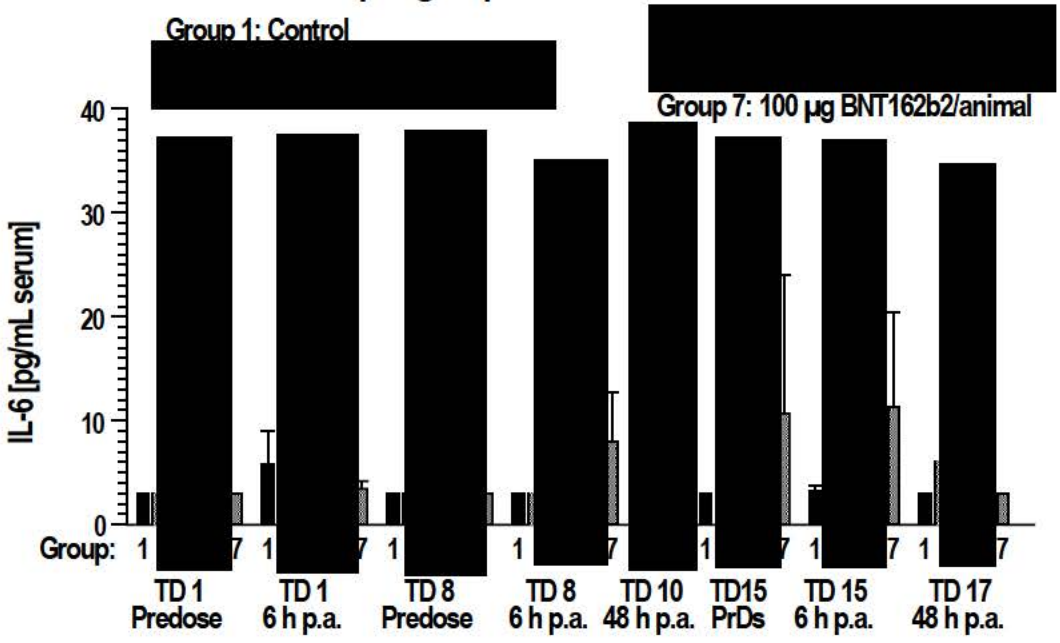


**Figure 11-1 IL-10 levels in serum of male rats
mean values per group and standard deviation**

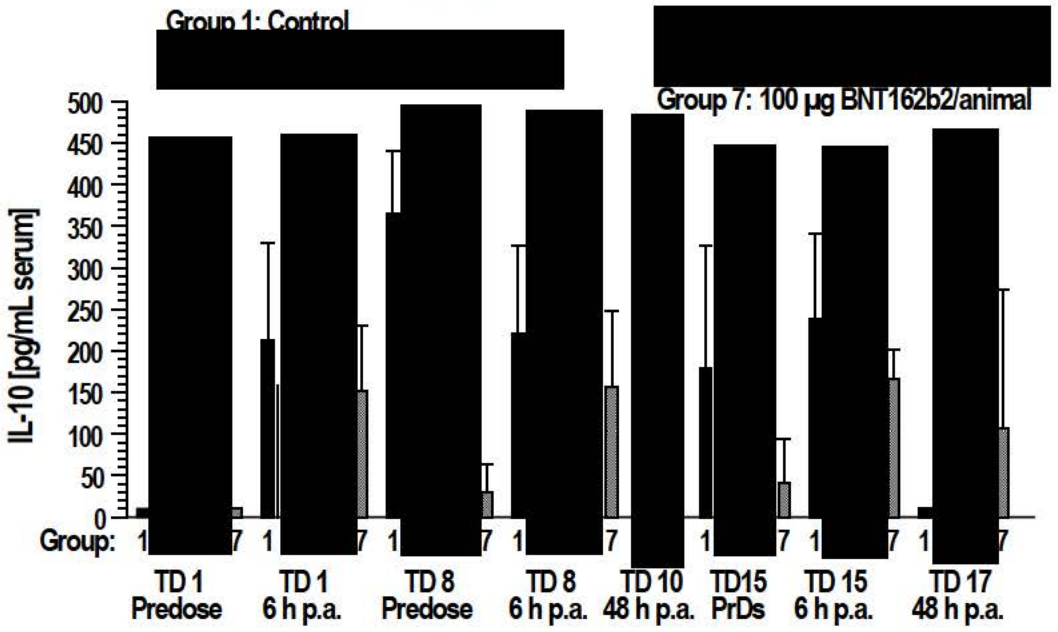
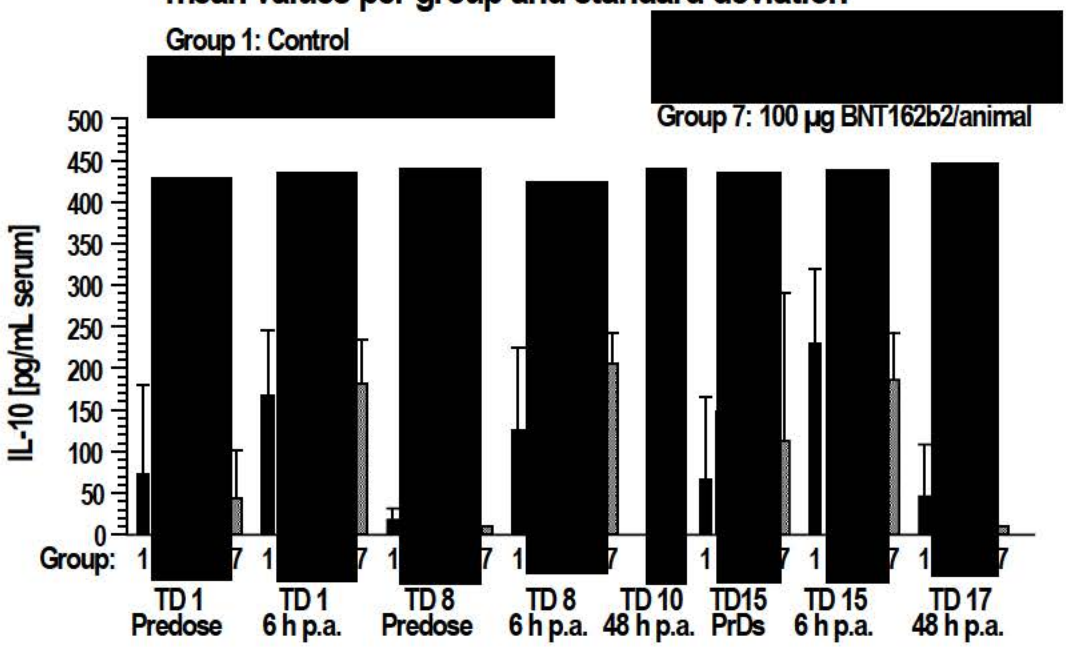


**Figure 11-2 IL-10 levels in serum of female rats
mean values per group and standard deviation**



4.11 Urinalysis

[redacted]

[redacted]BNT162b2 - Group 7

Treatment period and recovery period

Intramuscular treatment with [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15, [redacted] did not lead to any test item-related changes of the urinary parameters in the male and female animals compared to the respective control animals.

No test item-related changes were noted for the specific gravity, the pH value of the urine and the urine volume. The analyte concentrations of nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and haemoglobin were not influenced in a test item-related way in male and female animals. No test item-related changes were observed in the urine colour and the microscopically analysed urine sediments.

Text table 4-18: Statistically significant changes in urinary parameters considered not test item-related

Statistically significant changes in urinary parameters (refer to Table 10-1) in comparison to the control group considered not test item-related								
Urinary parameter	**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
Specific gravity	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
				[redacted]	[redacted]		[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
	7	4	100	m	17	+1.5	p ≤ 0.01	A
Urine volume	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
	7	4	100	m	17	-30.9	p ≤ 0.01	B

[redacted]
Test item 4: BNT162b2 - Group 7

m male
f female

A Change is within the limits of normal biological variation (with regard to the range covered by the control group) and without toxicological relevance.
B Change is due to the relative high or low value noted for the control group.

Group mean values of urinary parameters are presented in Table 10-1 (Urinalysis - Summary), individual data are listed in Table 10-2 (Urinalysis - Individual Data).

4.12 Immunogenicity assessment

The serum samples prepared from the blood collected at terminal dissection and recovery dissection (see Section 3.8.8) were analysed by [redacted] under the responsibility of the Sponsor. A summary from the analytical report forwarded to [redacted] is given following below.

'*The recorded data demonstrates that all BNT162 vaccine candidates elicited a SARS-CoV-2-S protein specific antibody response directed against the S1 domain and the RBD sub-domain.* [redacted] *BNT162b2 vaccines, antibody responses detected via ELISA increased over time and directly translated into neutralizing activity as seen in the VSV/SARS-CoV-2-S pseudovirus neutralization test. For those vaccine candidates, sera from animals with higher antigen-specific antibody titers also displayed more pronounced virus neutralization effect and, in case of modRNA based vaccines,* [redacted] *BNT162b2, exceeded the upper limit of quantification of the assay.*'

For details, refer to the analytical report 'Immunogenicity Assessment of [redacted] BNT162b2 [redacted] in Rat Serum after Repeated Intramuscular Administration' provided by [redacted] in Appendix 4.

4.13 Ophthalmological and auditory examinations

[redacted]

[redacted] BNT162b2 - Group 7

Treatment period and recovery period

The ophthalmological examination did not reveal any changes of the eyes and the optic region for the male and female animals following intramuscular treatment with [redacted] **100 µg BNT162b2/animal** on test days 1, 8, and 15, [redacted] at the end of the treatment period and at the end of the recvorey period.

There was no indication of any impairment to the auditory acuity through any treatment.

See Table 11 (Ophthalmological Examination) and Table 12 (Auditory Examination) for listings of individual findings.

4.14 Macroscopic *post mortem* findings

[redacted]

[redacted] **BNT162b2 - Group 7**

Terminal sacrifice

Test item-related findings were noted for all test items and all dose levels in male and female animals as given in the text tables following below.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted].

Text table 4-21: Incidences of test item-related macroscopic findings for the animals treated with [redacted] or BNT162b2

Incidences of test item-related macroscopic findings in male and female main study animals at necropsy at terminal sacrifice on [redacted] test day 17 (group 7) (refer to Table 13)				
Organ / Finding	[redacted]		**Group 7: 100 µg BNT162b2/animal**	
	[redacted]	[redacted]	**Males**	**Females**
External observation:				
- Injection site I and/or II thickened and/or incrusted	[redacted]	[redacted]	1/10	1/10
Injection site I and/or II (left/right):				
- Muscle(s) indurated or jellied / thickened / indurated / enlarged	[redacted]	[redacted]	7/10	9/10
Spleen:				
- Enlarged	[redacted]	[redacted]	2/10	7/10
Lymph node (iliac or iliac/renal):				
- Enlarged	[redacted]	[redacted]	5/10	6/10
Sciatic nerve (left):				
- Adhered to injection site I	[redacted]	[redacted]	0/10	3/10

.../... Number of animals affected per number of animals examined.

All systemic changes noted macroscopically are interpreted to be due to inflammation at the injection site and/or immune activation.

Recovery sacrifice

All macroscopic findings noted at the injection sites and for the spleen had subsided in all animals of all previously test item-treated groups at the end of the recovery period [redacted] test day 38 for all other groups)

Enlarged iliac lymph nodes were still noted for a few animals as follows:

[redacted] Group 7 (100 µg BNT162b2/animal): [redacted] One of 5 males, 3 of 5 females.

These findings are regarded to be related to the previous test item treatment.

Further findings in form of emphysematous lungs, a reddened thymus, an enlarged right testis, a dilated uterus, in some cases filled with a clear liquid, a prostate and seminal vesicles that were reduced in size, and enlarged adrenal glands were noted for individual male and female animals in the test item-treated groups and the control group at terminal sacrifice or at recovery sacrifice. Due to the isolated occurrence per finding, all of these findings are considered as spontaneous changes that are not test item-related.

The macroscopic findings of individual animals are listed in Table 13 (Macroscopic Post Mortem Findings).

4.15 Organ weights

[redacted]

[redacted]BNT162b2 - Group 7

Main study animals

In accordance with the macroscopic findings of enlarged spleens (see Section 4.11), increased relative and absolute spleen weights were noted for all test items at all dose levels in male and female animals as given in the text table following below.

Text table 4-22: Test item-related changes in spleen weights

Test item-related changes in spleen weights compared to the control group in % [redacted] test day 17/groups [redacted] and 7, refer to Table 14-1 and Table 15-1)			
Organ weight	[redacted]		
Organ weight	[redacted]	Group 7: 100 µg BNT162b2/animal	
		Males	Females
Rel.	[redacted]	+36.6**	+62.3**
Abs.	[redacted]	+25.2**	+60.8**

Rel. relative (to body weight)
Abs. absolute
*/** Statistically significant at $p \leq 0.05$ / $p \leq 0.01$ (based on numerical data, not on percent difference).
↑ Increase relative to study control range, but % difference not quantifiable due to lacking concurrent controls.

All other differences between any of the test item-treated groups [redacted] to 7 and the control group at the end of the treatment period [redacted] test day 17 for groups [redacted] and 7), or at the end of the recovery period [redacted] test day 38 for groups [redacted] and 7) are considered to be coincidental background changes within the normal range of biological variation.

Recovery period

The slightly increased spleen weights noted at the end of the treatment period for the male and female animals of all dose groups had subsided at the end of the recovery period. There were no noteworthy differences in the absolute and relative weights of any organ between the test item-treated animals and the control animals at the end of the recovery period [redacted] test day 38 for all other groups).

Statistically significant differences in organ weights compared to the control animals that are not considered to be test item-related are listed in the text table below.

Text table 4-23: Statistically significant organ weight changes considered not test item-related

Statistically significant differences in organ weights (refer to Table 14-1 and Table 15-1) in comparison to the control group considered not test item-related									
Organ		**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
Adrenal gland (left)	- rel.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		7	4	100	m	17	+23.4	p ≤ 0.05	A, D
	- abs.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
Brain	- rel.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
Epididymis (left)	- rel.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		7	4	100	m	17	+32.5	p ≤ 0.05	A, D
	- abs.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		7	4	100	m	17	+21.2	p ≤ 0.05	A, C
Epididymis (right)	- rel.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
		7	4	100	m	17	+33.9	p ≤ 0.01	A, B, D
	- abs.	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
		7	4	100	m	17	+22.7	p ≤ 0.05	A, C

- Text table continued on the next page -

Statistically significant differences in organ weights (refer to Table 14-1 and Table 15-1) in comparison to the control group considered not test item-related

Organ		Group	Test item no.#	Dose [µg/ animal]	Sex	Test day	Change [%]	Statistical significance	Reason
- Text table continued from previous page -									
Testis (left)	- rel.	[redacted]							
		7	4	100	m	17	+12.1	p ≤ 0.01	A
Testis (right	- rel.	[redacted]							
		7	4	100	m	17	+11.8	p ≤ 0.01	A
Heart	- rel.	[redacted]							
		7	4	100	m	17	+8.8	p ≤ 0.05	A
Kidney (left)	- rel.	[redacted]							
		7	4	100	f	17	+8.9	p ≤ 0.05	A, D
Kidney (right)	- rel.	[redacted]							
		7	4	100	f	17	+8.0	p ≤ 0.01	A, D
Liver	- rel.	[redacted]							
		7	4	100	f	17	+20.6	p ≤ 0.01	A, D
	- abs.	[redacted]							
		7	4	100	f	17	+19.2	p ≤ 0.01	A, C
Lungs	- rel.	[redacted]							
		7	4	100	f	17	+15.2	p ≤ 0.05	A, D
[redacted]	[redacted]	[redacted]							
[redacted]	[redacted]	[redacted]							
- Text table continued on the next page -									

Statistically significant differences in organ weights (refer to Table 14-1 and Table 15-1) in comparison to the control group considered not test item-related									
Organ		**Group**	**Test item no.#**	**Dose [µg/ animal]**	**Sex**	**Test day**	**Change [%]**	**Statistical significance**	**Reason**
- Text table continued from previous page -									
Thymus	- rel.	7	4	100	m	17	-21.3	$p \leq 0.05$	A, D
	- abs.	7	4	100	m	17	-27.9	$p \leq 0.01$	A

m male
f female

rel. relative
abs. absolute

A Change is within normal range of biological variation, without toxicological relevance.
B Change is due to the relatively low or high value noted for the control group.
C Change is related to the older age of the animals.
D Change is due to the slightly lower body weights of the respective test item treated animals compared to the control animals.
E Change is due to the slightly higher body weights of the respective test item treated animals compared to the control animals.

Relative organ weights are listed in Table 14-1 (Relative Organ Weights - Summary) and Table 14-2 (Relative Organ Weights - Individual Data). Absolute organ weights are listed in Table 15-1 (Absolute Organ Weights - Summary) and Table 15-2 (Absolute Organ Weights - Individual Data).

4.16 Histopathology

Terminal sacrifice

Test item-related microscopic findings at the end of dosing included 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. All microscopic findings were partially or fully recovered at the end of the 3 week recovery phase.

Test item-related injection site reactions were present in all groups and characterized by mostly moderate inflammation (up to marked) in males and moderate inflammation in females. The most severe findings were noted consistently in animals administered [redacted] **100 µg BNT162b2/animal**, [redacted]. The inflammation was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis, at the injection site. Injection site inflammation was associated with mostly moderate oedema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. [redacted] Injection site findings were partially recovered at the end of the 3-week recovery phase. 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). These findings were mostly recovered at the end of the 3-week recovery phase.

Test item-related findings in the draining (iliac) lymph node were characterized by increased cellularity of the follicular germinal centers and increased plasma cells (plasmacytosis) and were variably present in all groups.

Test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary hematopoiesis in the spleen were present in all groups.

A test item-related vacuolation of hepatocytes in the portal regions of the liver was present in all groups.

Test item-related findings were noted for all test items and all dose levels in male and female animals as given in the text tables following on the next pages.

[redacted]

[redacted]

[redacted]

[redacted]

Text table 4-26: Incidences of test item-related microscopic findings for the animals treated with [redacted] and BNT162b2

Incidences of test item-related microscopic findings in male and female main study animals after terminal sacrifice on [redacted] test day 17 (group 7)				
Organ / Finding	[redacted]		BNT162b2	
	[redacted]		Group 7: 100 µg/animal	
	[redacted]	[redacted]	Males	Females
Bone marrow:				
- Increased cellularity	[redacted]	[redacted]	10/10**	10/10**
Injection site I and/or II (left/right):				
- Fibrosis intramuscular/interstitial	[redacted]	[redacted]	10/10**	10/10**
- Fibrosis inter-/perimuscular			10/10**	10/10**
- Inflammation, mixed			10/10**	10/10**
- Myofiber degeneration			10/10**	10/10**
- Oedema, subcutis			10/10**	10/10**
- Oedema intramuscular/interstitial			10/10**	10/10**
- Oedema inter-/ perimuscular			10/10**	10/10**
- Hyperplasia, epidermis			9/10**	10/10**
Surrounding tissue of injection sites:				
Perineural tissue of sciatic nerve:				
- Inflammation (perineural)	[redacted]	[redacted]	10/10**	10/10**
Bone, os femoris with joint (surrounding tissue):				
- Inflammation	[redacted]	[redacted]	2/10	9/10**
Mammary gland (Interstitial tissue):				
- Inflammation	[redacted]	[redacted]	2/10	0/10
Lymph node (iliac):				
- Plasmacytosis	[redacted]	[redacted]	10/10**	10/10**
- Inflammation			9/10**	6/10*
- Increased cellularity, germinal center			10/10	10/10**
Skeletal muscle:				
- Infiltration, lymphohistiogranulocyt.	[redacted]	[redacted]	5/10*	0/10
Spleen:				
- Increased haematopoiesis	[redacted]	[redacted]	2/10	8/10**
Liver				
- Vacuolation, hepatocellular, periportal	[redacted]	[redacted]	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 ($p \leq 0.01$)

Recovery sacrifice

Most of the microscopic findings noted 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 at the end of the recovery period [redacted] test day 38 for all other groups). Some inflammatory lesions were still noted at the injection sites and the surrounding tissue of some animals, being less severe (minimal to mild) if not resolved; plasmacytosis in the iliac lymph node was less severe and present in fewer groups [redacted] 100 µg BNT162b2/animal) at the end of the 3-week recovery period, indicating partial or complete recovery.

The infiltration of macrophages in the iliac lymph nodes of previously treated recovery animals were regarded as consequence of phagocytosis relating to the inflammatory reactions at the injection sites.

Test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary hematopoiesis in the spleen was fully recovered at the end of the 3-week recovery phase.

Test item-related vacuolation of hepatocytes in the portal regions of the liver was fully recovered at the end of the 3-week recovery phase.

The incidence and the severity of the remaining findings were markedly reduced compared to the main study animals.

The complete Histopathology Report is given in Section 6.

HISTOPATHOLOGY REPORT

REPEAT-DOSE TOXICITY STUDY OF THREE LNP-FORMULATED RNA PLATFORMS ENCODING FOR VIRAL PROTEINS BY REPEATED INTRAMUSCULAR ADMINISTRATION TO WISTAR HAN RATS

[redacted] Project No.38166

27 June 2020

Veterinary Pathologist:
[redacted]

HISTOPATHOLOGY REPORT PAGE: I

[redacted] Study No. 38166

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

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Repeat-Dose Toxicity Study of
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Encoding for Viral Proteins by Repeated
Intramuscular Administration
to Wistar Han Rats

AUTHENTICATION

The undersigned hereby declares that the histopathology data in this report were compiled by him, and that they reflect accurately the primary data records.

[redacted]
Veterinary Pathologist

27 June 2020
Date

PRINCIPAL SECTION

METHODS

Group design for the histopathological evaluation

Text table 1: Group size and dose levels for the histopathological evaluation

Group	Dose level [µg/animal] (Test item / Control)	Number and sex of animals MS + RP	Animal no.	
			MS	RP
1	0 (Buffer) Control	10 + 5 m 10 + 5 f	1 - 10 16 - 25	11 - 15 26 - 30
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
7	100 (LNP modRNA Sp2) BNT162b2	10 + 5 m 10 + 5 f	181 - 190 196 - 205	191 - 195 206 - 210

MS: Main study
RP: Recovery period
m: male
f: female

The organs listed in section 3.8.10.2 of the main report of all animals of groups 1 to 7 were examined histologically after preparation of paraffin sections and haematoxylin-eosin staining.

The stained sections from all animals examined were prepared and provided by [redacted] The macroscopic findings were recorded and provided by [redacted]

Data compilation

The animal data and macroscopic observations were derived from descriptions recorded by [redacted], during the post mortem examination of each animal. The histopathological findings of the undersigned pathologist were recorded and calendared using the departmental computerized systems (Provantis® Integrated preclinical software, version 10.2.1, Instem LSS Ltd., United Kingdom).

The histopathological findings recorded in the organs/tissues are summarised in the tables 'Microscopic Findings by Incident' and 'Microscopic Findings by Severity'.

The 'Microscopic Findings by Incident' table lists the frequency of observations per group and sex as percentage of the affected animals per group.

The 'Microscopic Findings by Severity' table lists the severity of observations per group and sex (for severity grading see section 'Explanation of Codes and Symbols').

The 'Tabulated Animal Data' table lists the severity of observations for individual animals.

The 'Individual Animal Data' table comprises the animal data, the macroscopic observations and all microscopic observations of each animal.

The slides were evaluated in April, May and June 2020.

RESULTS AND CONCLUSION

Mortality

Main study / Recovery period

None of the male and female animals of groups 1 to 7 died or had to be sacrificed prematurely.

Macroscopic findings

[redacted]

[redacted] BNT162b2 - Group 7

Terminal sacrifice

Test item-related macroscopic findings at the end of dosing included injection site findings and increased spleen and draining lymph node (iliac) size. Increased spleen size correlated with increased absolute spleen weights and spleen:body weight ratios.

[redacted]

[redacted]	[redacted]			
	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]				
[redacted]				
[redacted]				
[redacted]				

[redacted]

[redacted]

[redacted]	[redacted]			
	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]				
[redacted]				
[redacted]				
[redacted]				

[redacted]

Text table 3: Incidences of test item-related macroscopic findings for the animals treated with [redacted] or BNT162b2

Incidences of test item-related macroscopic findings in male and female main study animals at necropsy at terminal sacrifice on test day 10 [redacted] or test day 17 (group 7)				
Organ / Finding	[redacted]		BNT162b2	
	[redacted]		Group 7: 100 µg/animal	
	[redacted]	[redacted]	Males	Females
External observation:				
- Injection site I and/or II thickened and/or incrusted	[redacted]	[redacted]	1/10	1/10
Injection site I and/or II (left/right):				
- Muscle(s) indurated or jellied / thickened / indurated / enlarged	[redacted]	[redacted]	7/10	9/10
Spleen:				
- Enlarged	[redacted]	[redacted]	2/10	7/10
Lymph node (iliac or iliac/renal):				
- Enlarged	[redacted]	[redacted]	5/10	6/10
Sciatic nerve (left):				
- adhered to injection site I	[redacted]	[redacted]	0/10	3/10

.../... number of animals affected per number of animals examined

Recovery sacrifice

All macroscopic findings noted at the injection sites and for the spleen had subsided in all animals of all previously test item-treated groups at the end of the recovery period [redacted] test day 38 for all other groups)

Enlarged iliac lymph nodes were still noted for a few animals as follows:

[redacted] [redacted]

Group 7 (100 µg BNT162b2/animal): One of 5 males, 3 of 5 females

These findings are regarded to be related to the previous test item treatment.

Further findings in form of emphysematous lungs, a reddened thymus, an enlarged right testis, a dilated uterus, in some cases filled with clear liquid, a prostate and seminal vesicles that were reduced in size, and enlarged adrenals were noted for individual male and female animals in the test item-treated groups and the control group at terminal sacrifice or at recovery sacrifice. Due to the isolated occurrence per finding, all of these findings are considered as spontaneous changes that are not test item-related.

Microscopic findings

Terminal sacrifice

Test item-related microscopic findings at the end of dosing included 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. All microscopic findings were partially or fully recovered at the end of the 3 week recovery phase.

Test item-related injection site reactions were present in all groups and characterized by mostly moderate inflammation (up to marked) in males and moderate inflammation in females. The most severe findings were noted consistently in animals administered [redacted] **100 µg BNT162b2/animal**, [redacted]. The inflammation was characterized by infiltrates of macrophages, granulocytes, and lymphocytes into the muscle, and variably into the dermis and subcutis, at the injection site. Injection site inflammation was associated with mostly moderate edema, mostly mild myofiber degeneration, occasional muscle necrosis, and mostly mild fibrosis. [redacted] Injection site findings were partially recovered at the end of the 3-week recovery phase. 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). These findings were mostly recovered at the end of the 3-week recovery phase.

Test item-related findings in the draining (iliac) lymph node were characterized by increased cellularity of the follicular germinal centers and increased plasma cells (plasmacytosis) and were variably present in all groups.

Test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary hematopoiesis in the spleen were present in all groups.

A test item-related vacuolation of hepatocytes in the portal regions of the liver was present in all groups.

A few minor microscopic changes were recorded for the organs examined in this study. The type, incidence and severity of all microscopic findings observed did not indicate any relationship to the treatment with the test item. All changes are regarded to be spontaneous in nature being within the normal background pathology commonly seen in rats of this strain and age.

Test item-related findings were noted for all test items and all dose levels in male and female animals as given in the text tables following on the next pages.

[redacted]

[redacted]

Text table 6: Incidences of test item-related microscopic findings for the animals treated with [redacted] and BNT162b2

Incidences of test item-related microscopic findings in male and female main study animals after terminal sacrifice on [redacted] test day 17 (group 7)				
Organ / Finding	[redacted]		**BNT162b2**	
	[redacted]		**Group 7: 100 µg/animal**	
	[redacted]	[redacted]	**Males**	**Females**
Bone marrow:				
- Increased cellularity	[redacted]	[redacted]	10/10**	10/10**
Injection site I and/or II (left/right):				
- Fibrosis intramuscular/interstitial	[redacted]	[redacted]	10/10**	10/10**
- Fibrosis inter-/perimuscular			10/10**	10/10**
- Inflammation, mixed			10/10**	10/10**
- Myofiber degeneration			10/10**	10/10**
- Edema, subcutis			10/10**	10/10**
- Edema intramuscular/interstitial			10/10**	10/10**
- Edema inter-/ perimuscular			10/10**	10/10**
- Hyperplasia, epidermis			9/10**	10/10**
Surrounding tissue of injection sites:				
Perineural tissue of sciatic nerve:				
- Inflammation (perineural)	[redacted]	[redacted]	10/10**	10/10**
Bone, os femoris with joint (surrounding tissue):				
- Inflammation	[redacted]	[redacted]	2/10	9/10**
Mammary gland (Interstitial tissue):				
- Inflammation	[redacted]	[redacted]	2/10	0/10
Lymph node (iliac):				
- Plasmacytosis	[redacted]	[redacted]	10/10**	10/10**
- Inflammation			9/10**	6/10*
- Increased cellularity, germinal center			10/10	10/10**
Skeletal muscle:				
- Infiltration, lymphohistiogranulocyt.	[redacted]	[redacted]	5/10*	0/10
Spleen:				
- Increased haematopoiesis	[redacted]	[redacted]	2/10	8/10**
Liver				
- Vacuolation, hepatocellular, periportal	[redacted]	[redacted]	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 ($p \leq 0.01$)

Recovery sacrifice

Most of the microscopic findings noted 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 had subsided in all animals of all previously test item-treated groups at the end of the recovery period [redacted] test day 38 for all other groups). Some inflammatory lesions were still noted at the injection sites and the surrounding tissue of some animals.

Test item-related minimal to mild increases in the cellularity of bone marrow and extramedullary hematopoiesis in the spleen were fully recovered at the end of the 3-week recovery phase.

Test item-related vacuolation of hepatocytes in the portal regions of the liver was fully recovered at the end of the 3-week recovery phase.

The incidence and the severity of the remaining findings were markedly reduced compared to the main study animals.

The infiltration of macrophages in the iliac lymph nodes of previously treated recovery animals were regarded as consequence of phagocytosis relating to the inflammatory reactions at the injection sites.

Discussion synopsis

Injection site inflammation, as well as inflammation in the adjacent tissues, was an anticipated response to an immune response to the administered test article. Inflammation was generally most severe in animals administered **100 µg** of [redacted] **BNT162b2/animal** at the end of dosing, [redacted] The inflammation was partially or fully resolved at the end of the recovery phase, indicating reversibility.

Increased cellularity of the germinal centers of the draining (iliac) lymph node and plasmacytosis is consistent with the anticipated immune activation by the test articles and inflammation at the injection site.

Increases in bone marrow cellularity (increased hematopoiesis) and extramedullary hematopoiesis in the spleen are consistent with a response to inflammation and immune responses induced by the test article.

Test item-related vacuolation of portal hepatocytes was present 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. The findings were fully reversed at the end of the recovery phase.

EXPLANATION OF CODES AND SYMBOLS

Tissue Result

N	tissue within normal histological limits
.	not recorded
+	tissue observation present
X	not examined

Grade

.	not recorded
1	minimal
2	mild
3	moderate
4	marked
#	different severities recorded, e.g. for the two parts of a paired organ
P	present - no grade or classification

Symbols

%	per cent
*	statistically significant (at $p \leq 0.05$, exact test of R. A. FISHER)
**	statistically significant (at $p \leq 0.01$, exact test of R. A. FISHER)
TGL	trackable gross lesion