Clinical Trial Protocol

EudraCT # 2021-001393-31
NCT05077267

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

15-Oct-2021

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1 General Information

1.1 Abbreviation

[redacted]	[redacted]
ADR	adverse drug reaction
AE	adverse event
ALT	alanine amino transferase
AP	alkaline phosphokinase
ACE2	angiotensin-converting enzyme 2
AESI	adverse event of special interest
AST	aspartate aminotransferase
BN	Bavarian Nordic
β-HCG	beta-human chorionic gonadotropin
BMI	body mass index
COVID-19	coronavirus disease 2019
CRA	clinical research associate
CRO	contract research organization
CTS	clinical trial site
cVLP	capsid virus-like particle
DMC	data monitoring committee
DS	drug safety
ECG	electrocardiogram
eCRF(s)	electronic case report form(s)
EDC	electronic data capture
EAP	end of active trial phase
ELISPOT	enzyme-linked immuno spot technique
ELISA	enzyme-linked immunosorbent assay
EU	European Union
EudraCT	European Clinical Trials Database
FU	follow-up
FDA	food and drug administration
GCP	good clinical practice
HBsAG	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IB	Investigator's Brochure
IEC	independent ethics committee
ICF	informed consent form
IRB	Institutional Review Board

ICH	International Conference on Harmonization
IM	intramuscular
IMP	investigational medicinal product
MedDRA	Medical Dictionary for Regulatory Activities
MP	medicinal product
NHP	nonhuman primate
n/N	number
PBMC	peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PV	pharmacovigilance
PI	principal investigator
QTc	QT interval corrected
RBD	receptor binding domain
SCR	screening visit
SADR	serious adverse drug reaction
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SOP	standard operating procedure
V	visit
WBC	white blood cells
WOCBP	women of childbearing potential

1.2 Definitions

active trial phase	The period from the first vaccination up to and including 1 month (28-35 days) after receiving the last trial vaccination. For Group 1 this includes V1-V7. For Groups 2 and 3, this includes V1-V4.
completed vaccination regimen	Completed vaccination regimen with an authorized SARS-CoV-2 vaccine. "Authorized" SARS-CoV2 vaccine refers to authorization status at SCR, i.e., subjects can be eligible if they previously received investigational vaccines that have since been authorized for emergency use or granted full market licensure. "Completed" includes full primary vaccination as described in the labeling of the initial vaccine, but also includes any mix/match series of 2 doses of any authorized COVID-19 vaccine, or a single dose of any authorized COVID-19 vaccine in subjects who previously had a confirmed COVID-19 infection.
end of active trial phase (EAP)	The visit at the end of active trial phase, approximately 28 to 35 days after the last vaccination. In the event of early withdrawal of a subject from the trial during the active trial period, the EAP visit will be the visit at which they collect the final safety endpoints for the trial.
last vaccination	For Group 1 (seronegative) the last vaccination is the second vaccination of their two-dose prime-boost regimen, scheduled to be received at V4. For Groups 2 and 3 (seropositive) the last vaccination is the single boost vaccination received at V1.
licensed SARS-CoV-2 vaccine	licensed SARS-CoV-2 vaccine refers to the license status at the time a subject is screened for the trial. A subject who received a SARS-CoV-2 vaccine during a clinical trial which has since been approved is eligible for the trial as the vaccine is presently considered licensed.

1.3 Protocol Synopsis

Title	An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects
Protocol Number	ABNCoV2-01
Clinical Phase	Phase 2
Sponsor	Bavarian Nordic A/S Philip Heymans Alle 3 2900 Hellerup Denmark
Trial Identifier	EudraCT # 2021-001393-31
Number of Sites and Countries	2 sites, Germany
Vaccination Dose and Schedule	Each subject will receive either 2 doses of 100 µg (Group 1), 1 dose of 100 µg (Group 2) or 1 dose of 50 µg (Group 3) ABNCoV2 vaccine. In the case of the two-dose regimen, vaccines are administered 4 weeks apart.
Route of Administration	ABNCoV2 vaccine is administered intramuscularly (IM) into the deltoid muscle of the upper arm (preferably the non-dominant arm).
Trial Duration	Approximately 17 weeks for initially seronegative subjects receiving the two-dose vaccination regimen (Group 1). Up to 104 weeks for initially seropositive subjects receiving 1 vaccination (Group 2 and Group 3).
Sample Size	A total of 210 healthy adult subjects will be enrolled into 3 groups: • Approx. 30 subjects determined to be seronegative for SARS-CoV-2 antibodies at screening (SCR) will be enrolled in Group 1. • Approx. 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 2 (100 µg). • Approx. 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 3 (50 µg).

Primary Objectives	To assess SARS-CoV-2 specific humoral immune responses of the ABNCoV2 vaccine in initially SARS-CoV-2 seronegative and seropositive subjects.
Secondary Objectives	To assess the safety and tolerability of the ABNCoV2 vaccine in adult seropositive and seronegative subjects.
Exploratory Objectives	To assess SARS-CoV-2 specific peak humoral immune responses after the prime-boost regimen (Group 1) or the booster dose (Group 2 and Group 3) of the ABNCoV2 vaccine. To explore SARS-CoV-2 specific cellular immune responses to the ABNCoV2 vaccine. To assess SARS-CoV-2 specific neutralizing immune responses against variant strains circulating at the time of analysis.
Primary Endpoints	SARS-CoV-2 neutralizing antibody titers at 2 weeks after the last vaccination, i.e., after the second vaccination in initially seronegative subjects and after the single boost vaccination in initially seropositive subjects.
Secondary Endpoints	Subjects reporting any SAEs or AESIs assessed as related to trial vaccine within 8 days after vaccination. Subjects reporting any Grade 3 or higher AEs assessed as related to trial vaccine within 8 days after vaccination.
Exploratory Endpoints	<u>Group 1 (seronegative subjects):</u> SARS-CoV-2 neutralizing antibody titers at 2 and 4 weeks post-prime vaccination and 1, 4, and 13 weeks post-boost vaccination. SARS-CoV-2 total antibody titers at all serum sampling time points. <u>Groups 2 and 3 (seropositive subjects):</u> SARS-CoV-2 neutralizing antibody titers at 1 week, 4 weeks, 3 months, 6 months, 1 year and 2 years after vaccination. SARS-CoV-2 total antibody titers at all serum sampling timepoints.

	Groups 1, 2 and 3 (all subjects): Cellular immune response, e.g., by Interferon-γ / Interleukin-4 ELISPOT, at 1 week after the last vaccination, i.e., after the second vaccination in initially seronegative subjects and after the single boost vaccination in initially seropositive subjects. SARS-CoV-2 neutralizing antibody titers against variant strains circulating at the time of analysis at 2 weeks after last vaccination.
Safety Endpoints	Subjects reporting any SAE or AESI, regardless of relationship, within 29 days after vaccination. Subjects reporting any Grade 3 or higher AEs assessed as related to trial vaccine within 29 days after vaccination. Subjects reporting solicited local AEs within 8 days after vaccination. Subjects reporting solicited general AEs within 8 days after vaccination.
Trial Design	A prior phase 1, dose ranging trial in healthy, seronegative adults evaluates safety and reactogenicity with doses up to 70µg, "*First-in-human Trial of the Coronavirus Virus-like Particle Subunit Vaccine ABNCoV2 in SARS-CoV-2-naïve Adult Volunteers in Good Health"* (COUGH-1; EudraCT no. 2020-004621-22, NCT04839146). For this Phase 2 trial ABNCoV2-01, in a run in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) will be vaccinated at 1 clinical trial site in a consecutive manner, with an at least 48 hours interval between the first and second subject of each group, then the second and third subject dosed on consecutive days, before opening up to full enrollment of the trial. Safety assessments will be based on solicited and unsolicited AE data (first week after first vaccination) evaluated by an independent Data Monitoring Committee (DMC). After a positive DMC recommendation, enrollment to the rest of Groups 1 and 2 of the trial will commence. Group 3 subjects will be enrolled after completion of Group 2 enrollment. This phase 2 trial will evaluate a homologous prime-boost regimen with 100 µg ABNCoV2 in initially seronegative adult subjects (Group 1), as determined by a qualitative test for SARS-CoV-2 antibodies, compared to a single boost vaccination of 100 µg (Group

<table>
<tr><td></td><td>2) or 50 µg (Group 3) of ABNCoV2 in initially seropositive subjects, as defined by a positive qualitative test for SARS-CoV-2 antibodies and either a history of SARS-CoV-2 vaccination or previous COVID-19 disease at least 90 days prior to enrollment (Table 1).

Table 1 Treatment Groups and Number of Subjects
<table><tr><th rowspan="2">Dose (/0.3 mL)</th><th colspan="2">Treatment Groups</th><th rowspan="2">Total N</th></tr><tr><th>Seronegative</th><th>Seropositive</th></tr><tr><td>100 µg</td><td>Group 1
30 subjects[a]</td><td>Group 2
90 subjects[a]</td><td>120</td></tr><tr><td>50 µg</td><td></td><td>Group 3
90 subjects</td><td>90</td></tr><tr><td colspan="3">Total</td><td>210</td></tr></table>N = number of subjects
[a] n = 3 of Group 1 and Group 2 (total 6 subjects) will be enrolled in the run-in phase before opening the trial to full enrollment.

For the seropositive subjects (Groups 2 and 3), the reason for seropositivity will be documented (e.g., previous COVID-19 disease, previous mRNA-based vaccination regimen, or previous Adenovirus-based vaccination regimen).

Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.</td></tr>
<tr><td>Trial Halting Criteria</td><td>A temporary halting or termination of the trial as a whole can be decided in case of:

• an SAE or serious AESI with an at least reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine

• an unexpected Grade 3 or higher systemic reaction or lab toxicity with at least a reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine

These parameters are not all-inclusive. Other AEs could occur that would trigger a DMC review. Any member of the DMC, the PI and/or the BN Medical Monitor could request a DMC review based on any observation.</td></tr>
</table>

	If an event fulfilling the trial halting criteria reaches the investigator's attention, the investigator has the responsibility to alert the Clinical Safety and Pharmacovigilance Department and Medical Monitor within 24 hours and provide comprehensive documentation of the event.
Inclusion Criteria	1. Subjects ≥18 years of age at SCR. 2. Seronegative (Group 1): negative qualitative test for SARS-CoV-2 antibodies at SCR. Seropositive (Group 2 and Group 3): Previous COVID-19 disease or previously completed vaccination regimen with an authorized SARS-CoV-2 vaccine at least 90 days before planned trial vaccination, and a positive qualitative test for SARS-CoV-2 antibodies at SCR. "Authorized" SARS-CoV-2 vaccine refers to authorization status at SCR, i.e., subjects can be eligible if they previously received investigational vaccines that have since been authorized for emergency use or granted full market licensure. Receipt of a single dose of an authorized COVID-19 vaccine regimen in subjects with a previous diagnosis of COVID-19 or a mix/match series of 2 doses of any authorized COVID-19 vaccine will be considered as a completed vaccination. 3. General good health, without acute medical illness, physical exam findings, or laboratory abnormalities, as determined by the investigator. 4. Prior to performance of any trial specific procedures, the subject has read, signed and dated an informed consent form (ICF), having been advised of the risks and benefits of the trial in a language understood by the subject. 5. Body mass index (BMI) ≥18.5 and <40. 6. Female subjects of childbearing potential (WOCBP) must agree to the use of an effective method of birth control from at least 30 days prior to administration of the vaccine until 30 days after the vaccination. Male subjects who are sexually active with a WOCBP must agree to the use of an effective method of birth control from the day of administration of the vaccine until 30 days after the vaccination. A woman is considered of childbearing potential unless post-menopausal (defined as ≥12 months without a menstrual period at SCR) or surgically sterilized (bilateral oophorectomy, bilateral tubal ligation, hysterectomy). Acceptable contraception methods are

	restricted to abstinence (abstinence only acceptable if refraining from heterosexual intercourse during the entire period of 30 days prior to administration of the vaccine until 30 days after the vaccination), double barrier contraceptives, vasectomy, intrauterine contraceptive devices or licensed hormonal products. 7. WOCBP must have a negative serum pregnancy test at SCR. 8. Negative human immunodeficiency virus antibody test (anti-HIV), negative hepatitis B surface antigen (HBsAG) and negative antibody to hepatitis C virus (HCV).
Exclusion Criteria	1. Group 1 only: History of COVID-19 infection or previous vaccination with a licensed or candidate SARS-CoV-2 vaccine, or positive qualitative test for SARS-CoV-2 antibodies at SCR. Groups 2 and 3 only: History of COVID-19 infection and subsequent receipt of more than one licensed or candidate SARS-CoV-2 vaccine. 2. Positive test for SARS-CoV-2 infection at SCR. 3. Pregnant or breastfeeding women. 4. Subject has an acute or chronic medical condition that, in the opinion of the investigator, would render the trial procedures unsafe or would interfere with the evaluation of the responses. 5. History of or active autoimmune disease. History of Guillain-Barré syndrome or Reye's syndrome. Persons with vitiligo or thyroid disease taking thyroid replacement are not excluded. 6. Known or suspected impairment of immunologic functions including, but not limited to, known immunodeficiency syndrome. 7. History of malignancy other than squamous cell or basal cell skin cancer, unless there has been surgical excision at least 6 months prior to SCR that is considered to have achieved cure. Subjects with history of skin cancer must not be vaccinated at the previous tumor site. 8. Laboratory parameters (such as complete blood count, serum biochemistry including aspartate aminotransferase [AST], alanine amino transferase [ALT], alkaline phosphokinase [AP], bilirubin, or creatinine values), pulse rate, blood pressure, or electrocardiogram (ECG) outside normal range at SCR and deemed clinically relevant by the investigator. 9. Clinically significant mental disorder not adequately controlled by medical treatment.

	10. Active or recent history (within 6 months before SCR) of chronic alcohol abuse, intravenous drug abuse, or nasal drug abuse. 11. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine. 12. History of anaphylaxis or severe allergic reaction to any vaccine. 13. Having received any vaccinations or planned vaccinations with a live vaccine within 30 days prior to or after trial vaccination. 14. Having received any vaccinations or planned vaccinations with an inactivated vaccine within 14 days prior to or after trial vaccination. 15. Recent blood donation (including platelets, plasma and red blood cells) within 4 weeks prior to SCR, or planned blood donations during the active trial phase. 16. Chronic systemic administration (defined as more than 14 days of >5 mg prednisone [or equivalent]/day), or any other immune-modifying drugs during a period starting 3 months prior to administration of the vaccine and ending 4 weeks after the last vaccination. The use of topical, inhaled, ophthalmic and nasal glucocorticoids is allowed. 17. Post organ transplant subjects, whether or not receiving chronic immunosuppressive therapy. 18. Administration or planned administration of immunoglobulins and/or any blood products during a period starting 3 months prior to administration of the vaccine and ending 4 weeks after the last vaccination. Receipt of packed red blood cells given for an emergency indication in an otherwise healthy person, and not required as ongoing treatment is not exclusionary (for example packed red blood cells given in emergency during an elective surgery). 19. Use of any investigational or non-registered drug or vaccine other than the trial vaccine within 30 days preceding the administration of trial vaccine, or planned administration of such a drug or vaccine throughout the trial. 20. Clinical trial site personnel involved in this trial.

1.4 Trial Schedule

Group 1 Two-dose Vaccination Schedule (Seronegative Subjects):

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	V7/EAP	FU1
Day / Visit +… Days	**-14--1**	**1**	**V1 +5-7**	**V1 +12-16**	**V1 +28-35**	**V4 +7-10**	**V4 +12-16**	**V4 +28-35**	**V4 +91-105**
Target week	**-2**	**0**	**1**	**2**	**4**	**5**	**6**	**8**	**17**
Informed consent	X								
Check incl./excl. criteria	X	X							
Medical History	X								
Complete physical exam[a] (body height and weight, BMI assessment)	X								
Evaluation of vital signs[a]	X	X	X	X	X	X	X	X	X
Targeted physical exam incl. auscultation of the heart and lung[a]		X	X	X	X	X	X	X	X
ECG[a]	X								
Recording of prior and concomitant medication	X	X	X	X	X	X	X	X	
Counseling on avoidance of pregnancy for WOCBP[b]	X	X			X				
AE/SAE/AESI recording	X[i]	X	X	X	X	X	X	X	X[c]
Pregnancy test for WOCBP[d]	X	X			X			X	
Obtaining blood for safety lab[a]	X			X			X	X[e]	
SARS-CoV-2 specific antibody test	X								
SARS-CoV-2 infection test (PCR)	X		(X)	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]
Hep-B, HCV, HIV test	X								

Visit (V)	SCR	V1	V2	V3	V4	V5	V6	V7/EAP	FU1
Day / Visit +… Days	**-14--1**	**1**	**V1 +5-7**	**V1 +12-16**	**V1 +28-35**	**V4 +7-10**	**V4 +12-16**	**V4 +28-35**	**V4 +91-105**
Target week	**-2**	**0**	**1**	**2**	**4**	**5**	**6**	**8**	**17**
Serum collection for antibody testing[g]		X		X	X	X	X	X	X
Collection of PBMC[g]		X				X			
Vaccine administration & subject observation (≥30 minutes)		X			X				
Recording of immediate AEs		X			X				
Handout of memory aid		X			X				
Collection of memory aid[h]			X			X			
Examination of injection site			X			X			

Abbreviations: AE = adverse event; AESI = adverse event of special interest; BMI = body mass index; EAP = end of active phase visit; ECG = electrocardiogram; FU = follow-up; Hep-B = hepatitis-B; HCV = hepatitis-C virus; HIV = human immune deficiency virus; PBMC = peripheral blood mononuclear cells; SAE = serious adverse event; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SCR = Screening; WOCBP = woman of childbearing potential;

X: mandatory; (X): if indicated/if applicable

[a] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits. In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition or respiratory disorders

[b] Review of acceptable contraceptive methods and recent menstrual history with WOCBP

[c] Only SAEs/AESIs will be collected during the follow-up period (FU1)

[d] At SCR, a serum test must be performed. At other visits, a urine pregnancy test will be performed

[e] Only for subjects who discontinued during the trial and coming for EAP visit to obtain final safety data (Section 4.7)

[f] At any time during the trial starting 2 weeks after vaccination if clinically indicated, e.g. in the presence of COVID-19 typical symptoms

[g] Any serum blood or PBMC samples must be taken before vaccination

[h] If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved

[i] AESIs are not collected until vaccine has been received at visit 1.

Group 2 and Group 3 One-dose Vaccination Schedule (Seropositive Subjects):

Visit (V)	SCR	V1	V2	V3	V4/EAP	FU1	FU2	FU3	FU4
Day / Visit +… Days	**-14--1**	**1**	**V1 +7-10**	**V1 +12-16**	**V1 +28-35**	**V1 +91-105**	**V1 +181-195**	**V1 +361-375**	**V1 +722-736**
Target week	**-2**	**0**	**1**	**2**	**4**	**13**	**26**	**52**	**104**
Informed consent	X								
Check incl./excl. criteria	X	X							
Medical History	X								
Complete physical exam[a] (body height and weight, BMI assessment)	X								
Evaluation of vital signs[a]	X	X	X	X	X	X			
Targeted physical exam incl. auscultation of the heart and lung[a]		X	X	X	X	X			
ECG[a]	X								
Recording of prior and concomitant medication	X	X	X	X	X	X			
Counseling on avoidance of pregnancy for WOCBP[b]	X	X							
AE/SAE/AESI recording	X[i]	X	X	X	X	X[c]	X[c]	X[c]	X[c]
Pregnancy test for WOCBP[d]	X	X			X				
Obtaining blood for safety lab[a]	X			X	X[e]				
SARS-CoV-2 specific antibody test	X								
SARS-CoV-2 infection test (PCR)	X		(X)	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]	(X)[f]
Hep-B, HCV, HIV test	X								

Visit (V)	SCR	V1	V2	V3	V4/EAP	FU1	FU2	FU3	FU4
Day / Visit +… Days	**-14--1**	**1**	**V1 +7-10**	**V1 +12-16**	**V1 +28-35**	**V1 +91-105**	**V1 +181-195**	**V1 +361-375**	**V1 +722-736**
Target week	**-2**	**0**	**1**	**2**	**4**	**13**	**26**	**52**	**104**
Serum collection for antibody testing[g]		X	X	X	X	X	X	X	X
Collection of PBMC[g]		X	X						
Vaccine administration & subject observation (≥30 minutes)		X							
Recording of immediate AEs		X							
Handout of memory aid		X							
Collection of memory aid[h]			X						
Examination of injection site			X						

Abbreviations: AE = adverse event; AESI = adverse event of special interest; BMI = body mass index; EAP = end of active phase visit; ECG = electrocardiogram; FU = follow-up; Hep-B = hepatitis-B, HCV = hepatitis C virus; HIV = human immune deficiency virus; PBMC = peripheral blood mononuclear cells; SAE = serious adverse event; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SCR = Screening; WOCBP = woman of childbearing potential.

X: mandatory; (X): if indicated/if applicable

[a] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition or respiratory disorders

[b] Review of acceptable contraceptive methods and recent menstrual history with WOCBP

[c] Only SAEs/AESIs will be collected during the follow-up period (FU1-FU4)

[d] At SCR, a serum test must be performed. At other visits, a urine pregnancy test will be performed

[e] Only for subjects who discontinued during the trial and coming for EAP visit to obtain final safety data (Section 4.7)

[f] At any time during the trial starting 2 weeks after vaccination if clinically indicated, e.g. in the presence of COVID-19 typical symptoms

[g] Any serum blood or PBMC samples must be taken before vaccination

[h] If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved

[i] AESIs are not collected until vaccine has been received at visit 1.

1.5 Responsibilities

Trial Number	ABNCoV2-01
Title	**An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects** EudraCT # 2021-001393-31
Sponsor	Bavarian Nordic A/S Philip Heymans Alle 3 2900 Hellerup Denmark
Phone (direct office) E-mail	[redacted] info@bavarian-nordic.com
Project Leader	[redacted]
Phone E-mail	
Medical Monitor	[redacted]
Phone E-mail	
Trial Statistician	[redacted]
Phone E-mail	
CRO Project Manager	[redacted]

	[redacted]
Phone E-mail	
Laboratory (Immunogenicity testing)	[redacted]
Phone E-mail	

2 Background Information and Scientific Rationale

2.1 Introduction to SARS-CoV-2 Virus

SARS-CoV-2 infection, which causes coronavirus disease 2019 (COVID-19), is an ongoing worldwide public health emergency. The SARS-CoV-2 virus is not only highly infectious, but infections may lead to humoral immune responses with low virus neutralizing capacity (Chen et al., 2020, Robbiani et al., 2020, Wu et al., 2020).

SARS-CoV-2 entry into cells occurs through interaction of the virus spike receptor binding domain (RBD) with the angiotensin-converting enzyme 2 (ACE2) protein on human cells. Upon binding, ACE2 acts as a receptor and human proteases are recruited to mediate cell entry (Shang et al., 2020a).

In convalescent COVID-19 patients, despite generally low humoral responses, neutralizing antibodies specific to the RBD of SARS-CoV-2 are recurrently found (Robbianiet al., 2020). Interestingly, SARS-CoV-2 may be more effective in evading an effective immune response than SARS-CoV-2 (the coronavirus that causes SARS) because the SARS-CoV-2 spike more frequently assumes a conformational state that hides the spike RBD (Shang et al., 2020b, Walls et al., 2020, Wrapp et al., 2020). Therefore, displaying the spike RBD of SARS-CoV-2 in a manner that facilitates recognition by the immune system is likely to be protective against COVID-19.

2.2 ABNCoV2 Vaccine

[redacted]

2.3 Summary of Nonclinical Studies with ABNCoV2 Vaccine

The immunogenicity of ABNCoV2 has been demonstrated in mice (Fougeroux et al., 2021) and in non-human primates (NHP). A single intramuscular (IM) administration of 100 µgABNCoV2 induced SARS-CoV-2 neutralizing antibodies in NHP at comparable levels as found in human convalescent samples, while neutralizing antibody titers induced by 15 µg ABNCoV2 remained below convalescent levels. A second administration of 15 or 100 µg ABNCoV2 led to a >50-fold increase in titers. While 15 µg ABNCoV2 administered 2-times to NHP resulted in significantly reduced lung viral load after SARS-CoV-2 challenge compared to nonvaccinated control animals, the higher dose of 100 µg ABNCoV2 administered 2-times was more effective in that the majority of animals were completely free of virus in lung and also in nasal swabs, demonstrating complete protection. Therefore, the high dose administered 2-times has been chosen to be evaluated in seronegative subjects in the phase 2 trial (a single high dose boost regimen will be administered to seropositive subjects).

The safety and good tolerability of 100 µg ABNCoV2 (adjuvanted in a squalene in water emulsion) administered 2- or 3-times in rabbits was shown in a Good Laboratory Practice (GLP) compliant repeat dose toxicity and local tolerance study, that did not raise any safety concerns for human use.

The ABNCoV2 dose administered to rabbits translates to a human equivalent dose based on body surface area (FDA, 2005) of 774 µg for a 60 kg human; this provides a safety margin of >7x higher than the 100 µg human dose. Even when taking into account an offset between laboratories using different reagents to determine the ABNCoV2 protein concentration, a potentially lower dose of 70 µg instead of 100 µg used in the toxicity study translates to a human equivalent dose of 542 µg for a 60 kg human and still provides a safety margin of >5x higher than the 100 µg human dose in this phase 2 trial. Further, the number of vaccine applications (3) was in excess of the 1-2 administrations planned in this phase 2 trial, thereby providing an additional safety margin.

See further details in the current effective ABNCoV2 IB.

2.4 Clinical Trial Data with ABNCoV2 Vaccine

To date, no clinical trials with ABNCoV2 vaccine have been completed. A first in human trial is currently ongoing (COUGH-1, EudraCT no. 2020-004621-22/ NCT04839146), and has recruited first subjects in mid-March 2021. In a predefined dose escalation, SARS-CoV-2 naïve subjects will receive up to 70 µg of the ABNCoV2 vaccine in this phase 1 trial. Preliminary safety results in 45 subjects dosed up to 70 µg did not show any relevant safety findings. No final results from this trial are available yet. For further dose justification, please refer to Section 2.3.

2.5 Rationale

SARS-CoV-2 infection, which causes COVID-19, is an ongoing worldwide public health emergency. The SARS-CoV-2 virus is not only highly infectious, but infections may lead to humoral immune responses with low virus neutralizing capacity (Chen et al., 2020, Robbianiet al., 2020, Wu et al., 2020).

Despite the recent approval of several SARS-CoV-2 vaccines using different technologies, such as mRNA-based or adenovirus-vectored vaccines, there is still substantial spread of COVID-19 disease and related disease burdens. These burdens include fatal outcomes as well as the need for continued lockdown or social distancing measures, causing a high economic and societal impact.

The recent emergence of variant strains with yet unknown susceptibility to the currently available vaccines highlights the potential need for continuous adaptation of vaccines and the necessity to administer re-vaccinations (boosts) in populations considered at relevant risk.

Based on the assumption that large parts of the population will obtain at least an initial SARS-CoV-2 vaccination regimen in the coming months, potential medical need is expected to be related to the availability of boost vaccinations for seropositive subjects in order to maintain or adapt immunogenicity to continuously circulating strains of SARS-CoV-2.

This trial will, therefore, evaluate an initial two-dose vaccination regimen of ABNCoV2 in seronegative subjects as well as single dose boost regimens at two different strengths (50 ug and 100 ug/dose) of ABNCoV2 in seropositive subjects. This will provide meaningful insights into the safety, tolerability and immunogenicity of both vaccination regimens, and will confirm whether a single dose boost regimen in seropositive subjects will elicit a comparably high immune response as expected for the two-dose regimen based on the available nonclinical data.

2.6 Trial Population

Women and men of any ethnicity aged ≥18 years who meet all the inclusion and none of the exclusion criteria are eligible for enrollment into this trial.

2.7 Risk/Benefit Assessment

2.8 Potential Risk

Blood drawing may cause discomfort, bruising, light-headedness or fainting. Rarely, a blood draw may result in infection at the site of venipuncture.

Based on the available data obtained in the nonclinical rabbit toxicology study, the dose levels planned for this phase 2 trial are not expected to cause any major risks.

As with all vaccines, there is a risk of an allergic reaction or an anaphylactic event. Trial site staff will observe subjects for at least 30 minutes after each vaccination and, in the event that a severe allergic reaction and/or dyspnoea might occur, appropriate medical treatment and supervision will be readily available.

Based on available data from COUGH-1 study, there have been no SAEs reported.

As with all vaccines, there is a risk of temporary mild to moderate injection site reactions, such as injection site pain and/or tenderness, erythema, swelling, pruritus or induration. The above mentioned were reported most commonly in the COUGH-1 study following both doses of study vaccine. Also, systemic inflammatory reactions including flu-like symptoms such as fever, headache, nausea, muscle pain, chills or fatigue can occur. Headache, chills and fatigue were reported most commonly in the COUGH-1 study after first dose of study vaccine. After the second dose of study vaccine, myalgia was mostly commonly reported, followed by nausea, hyperhidrosis and a general feeling of warmth without fever.

2.9 Benefit

There will be no direct benefit to the trial subjects. Trial subjects will contribute significantly to the development of a vaccine targeting COVID-19 disease. Subjects might potentially acquire protection against SARS-CoV-2 virus. However, it cannot be said if the vaccine is efficacious against the COVID-19 disease in humans, as efficacy data available for the ABNCoV2 vaccine have so far been obtained in animal models only. Analysis of the samples collected will not directly benefit the subject.

3 Objectives

Please refer to Protocol Synopsis Section 1.3.

4 Trial Design

4.1 Experimental Design

The trial is designed as an open label phase 2 trial to evaluate safety, tolerability and immunogenicity of the ABNCoV2 vaccine after IM application. The trial will evaluate a homologous prime-boost regimen with 100 µg ABNCoV2 in initially seronegative adult subjects (Group 1), as determined by a qualitative test for SARS-CoV-2 antibodies, compared to a single boost vaccination with either 100 µg or 50 µg dose of ABNCoV2 in initially seropositive subjects (Groups 2 and 3), as defined by a positive qualitative test for SARS-CoV-2 antibodies and a history of SARS-CoV-2 vaccination or previous COVID-19 disease at least 90 days prior to enrollment (Table 1).

In total, approximately 210 subjects will be enrolled in this trial. Recruitment will start with a run-in phase of 6 subjects (3 in each of Groups 1 and 2, respectively, at least 48 hours interval between the first and second subject in each group, the second and third subject dosed on consecutive days) before opening up enrollment to both treatment groups. Safety assessments will be based on solicited and unsolicited AE data evaluated by an independent Data Monitoring Committee (DMC) who will review the safety information collected at the Week 1 Visit (V2). After a positive DMC recommendation for each of the groups, enrollment of the rest of Groups 1 and 2 of the trial will commence.

Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.

4.2 Description of Trial Procedures

The trial procedures will be conducted according to the trial procedure schedule (Section 1.4) and as described on the following pages. Visits must be scheduled within the protocol-allowed visit windows.

4.3 Screening Visit

All subjects must be thoroughly informed about all aspects of the trial (e.g., trial visit schedule, required evaluations and procedures, risks and benefits) as described in the informed consent form (ICF). Written informed consent must be obtained according to local requirements before any trial-related assessments may be carried out.

After the ICF has been collected, subjects will enter a screening period of up to 14 days before the first vaccination. Screening procedures are listed in the trial procedure schedule in Section 1.4.

If a subject has been screened and cannot be vaccinated because of a certain transient condition (e.g., abnormal lab value due to an acute condition or a missing lab evaluation due to mishandling of the sample), the respective test(s) should be repeated. The re-test must be performed within the 14-day window started by the initial SCR visit.

If a subject cannot be vaccinated due to other circumstances (e.g., completion of a wash-out period for a medication or vaccine not allowed during the trial) leading to a delay over the 14-day window, a complete re-screening must be performed and a new subject number will be assigned. The clock then re-starts at the new SCR visit within 14 days before first vaccination. A subject may be fully re-screened only once.

4.4 Active Trial Phase

After successfully passing the screening evaluations, the eligible volunteers can enter the active trial phase starting with V1 and ending at either V4 (Group 2 and Group 3, single boost vaccination), V7 (Group 1, two-dose prime-boost vaccination schedule), or premature discontinuation.

The procedure is described further in the Protocol Synopsis (Section 1.3).

The procedures performed at V1 and all following visits are listed in the trial procedure schedule in Section 1.4. Collection of immunogenicity samples and all other examinations listed above the vaccination event in the trial schedule must always be performed prior to vaccine administration.

At V1/Day 1 each subject in Groups 1 and 2 will receive 1 dose of 100 µg ABNCoV2 vaccine, and Group 3 will receive 1 dose of 50 µg ABNCoV2 vaccine administered IM into the deltoid muscle of the upper arm (preferably the non-dominant arm). For Group 1 only, at V4 a second dose of 100 µg ABNCoV2 vaccine will be administered IM into the deltoid muscle of the upper arm (preferably the non-dominant arm).

Following the vaccination subjects will be kept under close observation at the clinical trial site (CTS) for at least 30 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following vaccine administration. Any AEs occurring during or after vaccine administration and observation period will be recorded immediately.

Solicited local and general AEs will be collected on a memory aid completed daily by the subject for an 8-day period, beginning with the day of vaccination. For each subject in Group 1, two memory aids will be collected (one for each of the two vaccinations). The memory aid will be returned to the clinic staff at the following visit. If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved (Section 8.13). Unsolicited AEs will be assessed as described in Section 8.12.

4.5 Follow-Up Phase

To monitor long-term safety and immunogenicity, all subjects in Groups 1, 2 and 3 will come to the CTS for follow-up visits (FU1) 3 months after the last vaccination.

For additional immunogenicity and safety readouts, the Group 2 and Group 3 (initially seropositive) subjects, will come to the CTS for FU visits at 3 months (FU1), 6 months (FU2), 1 year (FU3) and 2 years (FU4) after their vaccination.

The procedures performed at the FU Visits are listed in the trial procedure schedule in Section 1.4

4.6 Unscheduled Visit

Clinically indicated additional visits may be necessary between scheduled visits. Unscheduled visits may be performed, e.g., to repeat laboratory testing or physical exams due to a new development. Examinations performed at unscheduled visits will be documented in the source documents as well as in the respective electronic case report form (eCRF) sections for unscheduled visits.

4.7 Early Discontinuation

Reasons for early discontinuations:

A subject may be discontinued from the trial early for different reasons. The decision to discontinue early can be made by the investigator or by the subject. Reasons for early discontinuation of a subject may include, but are not limited to:

- An AE/SAE/AESI that, in the opinion of the investigator, makes it unsafe for the subject to receive a vaccination
- Pregnancy
- An anaphylactic reaction following the administration of any vaccine(s)
- Clinical need for concomitant or ancillary therapy not permitted in the trial as outlined in Section 8.8.
- Subject's receipt of an approved booster vaccination for SARS-CoV-2.
- Subject's request to discontinue (withdrawal of consent to participate).
- Subject unwilling or unable to comply with trial requirements.
- Any reason that, in the opinion of the investigator, requires or supports early discontinuation of a subject.

Handling of early discontinuations:

Each subject has the right to terminate their trial participation completely at any time for any reason, and the investigator may also terminate a subject's trial participation. Subjects whose trial participation is terminated during the active trial phase should undergo a concluding EAP visit, including safety laboratory testing and pregnancy test for WOCBP. The subject has the right to refuse the optional EAP visit, however, the CTS should make an effort to collect any safety data possible for the subject. If the subject consents, the 3-month FU visit (FU1) should still be performed to collect any potential SAEs/AESIs which occurred after the last vaccination. Subjects who discontinued after receiving trial vaccine will not be replaced.

4.8 Trial Duration

The total duration of subject participation in the trial will be up to 104 weeks (one-dose vaccination schedule) or 17 weeks (two-dose vaccinations schedule). The duration of the trial as a whole ultimately depends on the length of the recruitment period.

4.9 Data Monitoring Committee

The DMC is an independent board that oversees the safety of subjects participating in the trial. The members of the DMC are independent experts with experience in infectious diseases. The primary responsibility of the DMC is to review and evaluate the accumulated trial safety data and make recommendations to proceed to open enrollment for remaining subjects.

If an event occurs which fulfills the trial halting rules (see Section 4.10 for further details), the DMC will review the event in a timely manner and agree on a recommendation to halt, resume or terminate the trial participation of the affected subject(s) and/or the trial as a whole.

A separate charter will describe in detail the relevant operational procedures, communication pathways, roles and responsibilities of the DMC.

4.10 Trial Halting Rules

The Trial Halting Criteria are as follows:

A temporary halting or termination for the trial as a whole can be decided in case of:

- a SAE with an at least reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine
- an unexpected Grade 3 or higher systemic reaction or lab toxicity with at least a reasonable possibility of a causal relationship to the administration of ABNCoV2 vaccine

These parameters are not all-inclusive. Other AEs could occur that would trigger a DMC review. Any member of the DMC, the Principal Investigator (PI) and/or the Bavarian Nordic (BN) Medical Monitor or safety physician could request a DMC review based on any observation.

If an event fulfilling the trial halting criteria reaches the investigator's attention, the investigator has the responsibility to alert the Clinical Safety and Pharmacovigilance (PV) Department and Medical Monitors within 24 hours and provide comprehensive documentation of the event.

5 Selection of Subjects

The investigators will keep a log of subjects screened for the trial and provide the reason in case of exclusion. Information about every screened subject will be documented in the eCRF.

For subjects not fulfilling the eligibility criteria the minimum information documented in the eCRF is confirmation of the ICF signature, demographics and reason for screen failure.

5.1 Recruitment Procedure

Subjects will be recruited actively. Recruitment strategies will be evaluated by BN prior to IRB/IEC approval. Subjects identified as potential subjects in the trial will be provided with all the necessary information required to make an informed decision about their participation in the trial.

5.2 Inclusion Criteria

Please refer to Protocol Synopsis Section 1.3.

5.3 Exclusion Criteria

Please refer to Protocol Synopsis Section 1.3.

6 Investigational Medicinal Product

6.1 Production, Packaging and Labeling

ABNCoV2 drug product is released by Bavarian Nordic A/S, DK.

Address: Bavarian Nordic A/S
[redacted]

The packages and vials of ABNCoV2 vaccine are labeled according to regulatory requirements. Vials contain at least 100 µg in 0.3 mL.

For dilution buffer phosphate buffered saline (PBS) is used and released by Bavarian-Nordic A/S, DK. The packages and vials of dilution buffer are labeled according to regulatory requirements.

6.2 Shipment, Storage and Handling

Usage of the investigational medicinal product (IMP) is only allowed upon final approval of all shipment relevant paperwork by BN or its authorized designee. Only subjects enrolled in the trial may receive IMP.

ABNCoV2 vaccine and dilution buffer PBS (phosphate buffered saline) will be shipped separately and temperature controlled and monitored to the CTS. Once at the site, the package should be handed over to the personnel in charge of IMP preparation (e.g., the pharmacist). Site personnel are responsible for proper storage of IMP upon receipt. All IMP must be stored in a secure, environmentally controlled and monitored area in accordance with the labelled storage conditions with access limited to the Investigator and authorized site staff.

ABNCoV2 vaccine is shipped at -20°C and must be stored frozen at -20°C (±5°C).

PBS has to be shipped and stored refrigerated at +2°C to +8°C .Details on shipment, storage and handling of the IMP (and comparable information for the diluent to be used for Group 3) are provided in the pharmacy manual.

6.3 Preparation, Administration and Dosage

The preparation and administration of the vaccine will be performed by authorized personnel only. The vaccine is administered IM into the deltoid muscle of the upper arm (preferably the non-dominant arm).

Trial specific details on preparation, dilution (for Group 3) and administration of the ABNCoV2 vaccine are provided in the pharmacy manual.

6.4 Accountability and Disposal

Used (if allowed by institutional policy) and unused vials of all IMP need to be retained in a place with limited access until appropriate drug accountability has been performed. Drug accountability must be documented whenever the IMP is either prepared or administered.

BN will provide a Drug Accountability Log for recording receipt, dispensation, and destruction of IMPs (see separate pharmacy manual). Alternative systems used to track drug accountability are acceptable for use in the trial provided the aforementioned items are adequately captured and records are available for review during scheduled monitoring visits to the site.

After drug accountability has been performed, used and unused vials should either be returned to the designated drug depot, a vendor selected by BN or discarded according to local regulations.

Destruction or return of IMP must be agreed upon with BN and appropriately documented. Documentation should be reviewed and signed off by the pharmacist and Clinical Research Associate (CRA) assigned to monitor the site.

Sites are responsible for the proper destruction and disposal of used needles and syringes, which should be performed according to local regulations. If local disposal is not possible, used clinical supplies may be returned to BN or to the designated drug depot after prior consultation with BN.

7 Assessment of Immunogenicity

The immunogenicity of the vaccine will be assessed by measuring humoral and cellular immune responses on collected serum and peripheral blood mononuclear cells (PBMC) samples.

Immunogenicity testing will be performed at [redacted] and at contracted laboratories, where applicable. Testing standard operating procedures (SOPs), effective at the time of testing will be filed in the electronic Trial Master File.

The procedures for collection, preparation, storage and shipment of specimens for immunogenicity testing (serum and PBMC) are specified in separate study-specific instructions, which will be provided to the investigators/CTS personnel as well as to the processing laboratories before recruitment commences. Additionally, investigators and CTS staff will be trained in the procedures during the investigator meeting/site initiation visit.

7.1 Humoral Immune Response

Serum samples will be collected from subjects as outlined in the trial procedure schedule in Section 1.4. Samples for immunogenicity testing will be obtained at the vaccination visits will be drawn prior to vaccination.

SARS-CoV-2 specific antibody responses will be assessed in trial subjects using enzyme-linked immunosorbent assay (ELISA; total antibodies) and neutralization tests (neutralizing antibodies).

7.2 Cellular Immune Response

PBMC samples will be collected as outlined in the trial procedure schedule in Section 1.4. Samples for immunogenicity testing obtained at the vaccination visits will be drawn prior to vaccination.

SARS-CoV-2 specific cellular responses will be assessed in vaccinated subjects, e.g., using an Interferon-γ / Interleukin-4 enzyme-linked immune spot technique (ELISPOT).

7.3 Future Use of Lab Specimen

Specimens remaining after completion of immunogenicity testing as per protocol will be stored for possible future research and analysis supporting the licensure path of ABNCoV2 vaccines. Future testing will facilitate the bridging of trial results to animal immunogenicity results and/or to immune response data collected from subjects vaccinated with other COVID-19 vaccines. Further, remaining samples might be used for assay development and controls. Subjects will be invited to consent to storage/future use of samples and will be informed about data protection measures. Specimens will be stored in BN's secured laboratory area [redacted], or at an external storage facility in a coded, pseudonymized manner to ensure data protection. Genetic testing will not be performed.

8 Safety and Reactogenicity

Safety will be monitored by collection of medical history, performing physical examinations including vital signs, when appropriate, electrocardiogram (ECG) and routine laboratory measurements (including pregnancy testing and counseling on avoidance of pregnancy for WOCBP) as well as by evaluating local and general solicited AEs and unsolicited AEs. AESIs are defined as per section 8.6 and are following the same reporting workflow as SAEs.

8.1 Definitions

8.2 Medical History

Symptoms, relevant laboratory findings and ongoing medical conditions present before and/or at SCR will be documented as medical history.

8.3 Adverse Events

AEs are defined as any untoward medical occurrence in a subject after signature of the ICF and which does not necessarily have a causal relationship associated with the administration of an IMP. Any new signs, symptoms, laboratory findings or changes in health starting after ICF signature are documented in the subjects' records and the AE section of the eCRF (for data collection requirements for screen failures see Section 5). AEs are recorded based on unsolicited and solicited questioning (Section 8.4 and 8.5).

8.4 Unsolicited AEs

Unsolicited AEs are defined as AEs which are not pre-listed on the memory aid card. Adverse events (e.g., feeling of ill-health, subjective symptoms and objective signs, intercurrent diseases, accidents, etc.) observed by the investigator and/or reported by the subject must be recorded in the eCRF regardless of the assessment of causality in relationship with the IMP/MP.

After the first vaccination, abnormal laboratory findings assessed as being clinically significant by the investigator are to be documented as AEs. In addition, after first vaccination abnormal laboratory findings fulfilling the Grade 3 or Grade 4 criteria according to the toxicity scale (Appendix 1) are to be documented as AEs in the eCRF, regardless of whether they are considered clinically relevant or not. For lab values fulfilling the Grade 3 or Grade 4 criteria, the decision to repeat the labs is left to the discretion of the PI. Toxicity grade and seriousness of an AE will be assessed separately, i.e., a Grade 3 or Grade 4 AE will not automatically be regarded as serious.

The investigator should ask the subjects if they have experienced any AEs since their last visit. All intercurrent diseases reported by the subject need to be recorded by the investigator in the appropriate section of the eCRF.

Any AEs or abnormal laboratory findings starting/reported during the screening phase, i.e., after informed consent is given but before the first vaccination, are considered as pre-treatment AEs.

8.5 Solicited AEs

In this clinical trial protocol solicited AEs are defined as all symptoms specifically listed in the memory aid provided to the subjects following each vaccination. After vaccination, the subjects are requested to monitor and record local symptoms (i.e., erythema, swelling, induration, pruritus and pain at the injection site) as well as general symptoms (i.e., body temperature increase/pyrexia, headache, chills, myalgia, nausea and fatigue) in the memory aid daily for the day of vaccination and the following 7 days (Days 1 to 8 following vaccination, 8-day period). If symptoms persist at Day 8, daily symptoms and temperature will continue to be measured and documented each day until resolved.

8.6 SAEs and AESIs

An SAE is any untoward medical occurrence that:

- Results in death
- Is life-threatening

  The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death, if it were more severe.
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

- Is an otherwise important medical event, e.g., leads to suspicion of transmission of an infectious agent.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Adverse Events of Special Interest (AESIs) are adopted as per the Safety Platform for Emergency vACcines (SPEAC) Project generated list of AESI for safety monitoring (accessible via https://brightoncollaboration.us/covid-19/) based on the following criteria:

1) known association with immunization or a specific vaccine platform;

2) theoretical association based on animal models;

3) occurrence during wild-type disease as a result of viral replication and/or immunopathogenesis

This list is updated on a quarterly basis and the current effective list of AESIs is applicable for this trial.

All AEs are collected from signing of ICF, however should not be considered by investigator to be an AESI unless occurring since receipt of first vaccination.

8.7 Relevant Medical History

Relevant medical history will be self-reported and documented at SCR and will focus particularly on any important diseases and in case of infections or tumors, the pathogen involved or the pathological diagnosis, if available. In particular, subjects with prior COVID-19 infection need to include the infection and dates in the medical history section of the eCRF. Additionally, laboratory reports need to be documented during SCR. All ongoing conditions for which the subjects are taking medications, and/or which may exacerbate during the trial period should be considered as important. Special attention should be given to history of prior allergic reactions, especially to vaccines.

8.8 Prior and Concomitant Medication

All concomitant (ongoing) medication except homeopathic substances and dietary supplements must be recorded in the subject's medical record and in the eCRF including information about the indication, dosage regimen, and the onset and end of treatment.

The following medications taken within 3 months prior to SCR will also be recorded in the eCRF and the subjects medical record: vaccines (e.g., influenza/pneumococcal), corticosteroids (via any route of administration), other immune-modulating drugs, immunoglobulin and/or any blood products, investigational drugs and depot preparations which are still active at the date of SCR. In addition, subjects with previous SARS-CoV-2 vaccination will include the specific vaccine received by dose and date in the eCRF, regardless of the time elapsed prior to SCR.

8.9 Prohibited Medications

Prohibited medication or medication where washout periods need to be adhered to are (see also eligibility criteria in Section 1.3):

- Vaccination with any licensed live vaccine within 30 days prior to first or after last trial vaccination or any licensed inactivated vaccine within 14 days prior to first or after last trial vaccination.
- Start of chronic systemic administration (defined as more than 14 days of > 5 mg prednisone [or equivalent]) per day or any other systemic use of immune-modifying drugs during a period starting from 3 months prior to first administration of the trial vaccine and ending at the last visit of the active trial phase. The use of topical, inhaled, ophthalmic and nasal corticoids will be permitted.
- Administration of immunoglobulins and/or any blood products during a period starting from 3 months prior to first administration of the trial vaccine and ending at the last visit of the active trial phase.
- Use of any investigational or non-registered drug or vaccine other than the trial vaccine within 30 days preceding the administration of the trial vaccine, or planned administration of such a drug during the trial period until the FU Visit 3 months after the last vaccination visit for Group 1, or 24 months after last vaccination visit for Groups 2 and 3.
- In case official guidelines require booster vaccinations to maintain valid COVID-19 vaccination certificates, approved booster vaccinations for SARS-CoV-2 are allowed for Groups 2 and 3 within the follow-up period, however subjects will be withdrawn from further follow up.

8.10 Physical Examination

Complete physical examination

A complete physical examination (excluding breast, genital, and rectal examinations) will be performed at SCR. The examination includes a review of major organ systems as well as body height and weight. The examination should be directed at finding evidence of any infections, tumors and lymphadenopathy (a grading scale for lymphadenopathy is included in Appendix 2). In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition or respiratory disorders.

Any clinically significant findings at the baseline physical examination will be recorded as medical history events. The only data captured in the eCRF for the physical examination itself will be the date it was performed. Any new or worsening clinically significant findings post-treatment will be captured as AEs.

Targeted physical examination

A targeted physical examination, guided by any signs or symptoms previously identified or any new symptoms that the subject has experienced since the last visit, is required according to the trial schedule (Section 1.4). In addition, auscultation of the heart and lungs will be performed to check specifically for signs of any heart condition.

Clinically significant findings from the active trial phase physical examinations will be recorded as AEs.

8.11 Vital Signs

At SCR and every other trial visit as defined in the trial schedule (Section 1.4), blood pressure and pulse rate will be taken after the subject has been sitting upright for approximately 2 minutes. Body temperature will be measured orally. Height and weight will be collected at SCR.

8.12 Unsolicited AEs

All intercurrent diseases reported when the investigator actively inquires the subject will be documented and all required details (e.g., start and stop date, intensity) will be assessed. Unsolicited AEs will be reported in the subject's medical record and respective section of the eCRF (requirements for screen failures see Section 12.1).

Unsolicited AEs will be assessed and documented from ICF signature through EAP, and if ongoing at that time followed until resolution or until the subject's last trial visit, at the latest. SAEs and AESIs will be collected through the end of the trial, including during the FU period.

SAEs and AESIs will be assessed and documented at all trial visits. (S)AEs would only be considered as AESIs once subject has received first vaccination. SAEs and AESIs will be followed-up until resolution or achievement of stable clinical conditions.

Assessment of Intensity

For all unsolicited AEs not represented in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, 2007), the maximum intensity will be based on the following descriptions:

Grade 1	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with daily activities.
Grade 2	An AE which is sufficiently discomforting to interfere with daily activities, but does not require medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose).
Grade 3	An AE which prevents daily activities and which requires medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose)
Grade 4	Life-threatening, or disabling.

Assessment of Causality

The relationship between the occurrence of an AE and the IMP will be assessed using the categories presented below. For expedited reporting and all other purposes, the categories "none" and "unlikely" will represent no evidence or argument to suggest a causal relationship, while "possible", "probable" and "definite" will be seen to convey that there is evidence or argument to suggest a causal relationship. To ensure reporting, all AEs without a causality assessment from the investigator will preliminarily be classified as "possible".

In terms of binary causality assessments unlikely and not related in clinical trials is lumped to "not related" whereas other causality assessments as per below are lumped to "related".

Not related	The time interval between administration of the IMP and the occurrence or worsening of the AE rules out a relationship, and/or alternative cause is established and there is no evidence of a (concomitant) causal connection with or worsening caused by the IMP.

Unlikely The time interval between administration of the IMP and the occurrence or worsening of the AE makes a causal relationship unlikely, and/or the known effects of the IMP or substance class provide no indication of a (concomitant) causal connection with or worsening caused by the IMP and there is another cause which serves as an adequate explanation, and/or although the known effects of the IMP or substance class make it possible to derive a plausible causal chain with regard to a (concomitant) causal connection or worsening, however, another cause is considerably more likely, and/or another cause of the AE has been identified and a (concomitant) causal connection with or worsening caused by the IMP is unlikely.

Possible A plausible causal chain with regard to a (concomitant) causal connection with / worsening of the AE can be derived from the pharmacological properties of the IMP or substance class. However, other approximately equally likely causes are known, or although the pharmacological properties of the IMP or substance class provide no indication of a (concomitant) causal connection with / worsening of the AE, there is no other known cause which provides an adequate explanation.

Probable The pharmacological properties of the IMP or substance class, and/or the course of the AE after discontinuation of the IMP and possible subsequent re-exposure, and/or specific findings (e.g., positive allergy test or antibodies against the IMP / metabolites) suggest a (concomitant) causal connection with / worsening of the AE resulting from the IMP, however another cause cannot completely be ruled out.

Definite The pharmacological properties of the IMP or substance class and/or the course of the AE after discontinuation of the IMP and possible subsequent re-exposure, and/or specific findings (e.g., positive allergy test or antibodies against the IMP / metabolites) definitely indicate that there is a (concomitant) causal connection with / worsening of the AE resulting from the IMP and there are no indications of other causes.

8.13 Solicited AEs

After the vaccination, subjects receive a memory aid to record solicited local and general AEs most likely to occur on the day of vaccination and the following 7 days (Days 1 to 8 following vaccination, 8-day period).

All solicited symptoms observed after vaccination with details concerning the intensity and the course of the symptom should be documented in the memory aid. The CTS will collect the memory aid at the following visit, review the memory aid with the subject, and transfer the results to the eCRF and the subject's medical record. The investigator's assessment of the symptoms will also be recorded in the eCRF including causality (for solicited general AEs),

seriousness, outcome, action taken with vaccine, and any intervention required. Local and general symptoms still ongoing after 8 days will continue to be measured and documented each day until resolved.

To standardize procedures uniform rulers will be handed out to subjects for measurements of erythema, swelling and induration diameters, as will digital thermometers for oral measurements of body temperature.

In case of severe and unexpected local and/or general reactions, the subject should be instructed to contact the trial physician outside of scheduled trial visits.

8.14 Solicited Local AEs

The solicited local symptoms erythema, swelling, induration, pruritus and pain at the injection site are to be documented in the memory aid by the subjects.

Assessment of Intensity

Injection site erythema, swelling, and induration will be assessed based on the longest diameter as measured by the subject in mm and recorded in the memory aid. Subjects are asked to document the solicited local AEs in the memory aid as described in Table 2 below.

Table 2 Grading of Local Symptoms from the Subject's Memory Aid

MedDRA coded Preferred Term Local Adverse Events	Grade	Severity
Injection site erythema, Injection site swelling, and Injection site induration (longest diameter measured in mm)	0	0 mm
	1	<30 mm
	2	≥30 – <100 mm
	3	≥100 mm
Injection site pruritis	0	Absent
	1	Mild
	2	Moderate
	3	Severe
Injection site pain	0	Absent
	1	Painful on touch
	2	Painful when limb is moved
	3	Spontaneously painful/prevents normal activity

Assessment of Causality

Solicited local AEs are defined as being related to the vaccine.

8.15 Solicited General AEs

The solicited general symptoms body temperature, headache, myalgia, nausea, chills and fatigue are to be documented in the memory aid by the subjects.

Assessment of Intensity

Subjects are asked to document the solicited general AEs in the memory aid as described in Table 3 below. In the subject's memory aid, the grading of symptom intensity is described in basic, easily understood language based on the following descriptions:

Table 3 Grading of General Symptoms from the Subject's Memory Aid

MedDRA coded Preferred Term General Adverse Events	Grade	Severity
Body temperature (oral) *	0	<37.5°C (<99.5°F)
	1	≥37.5 – <38.0°C (≥99.5 – <100.4°F)
	2	≥38.0 – <39.0°C (≥100.4 – <102.2°F)
	3	≥39.0 – <40.0°C (≥102.2 – <104.0°F)
	4	≥40.0°C (≥104.0°F)
Headache, Myalgia, Nausea, Chills and Fatigue	0	None
	1	Mild: easily tolerated, minimal discomfort and no interference with daily activity
	2	Moderate: Some interference with daily activity
	3	Severe: Prevents daily activity

*Pyrexia is defined as oral temperature ≥38.0°C (≥100.4°F)

Assessment of Causality

Causal relationship between solicited general AEs and the vaccine will be assessed by the investigator using the same categories as for unsolicited AEs (see Section 8.12).

8.16 Safety Laboratory Measurements

The intensity of laboratory/systemic toxicities measured quantitatively will be graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, 2007). The laboratory values provided in this toxicity grading scale serve as guidelines and are dependent upon institutional parameters. Institutional specific toxicity gradings will be included in site specific manuals as needed.

Safety laboratory is determined at SCR and V3 (one-dose vaccination schedule) or at SCR, V3 and V6 (two-dose vaccination schedule) and at any other visit(s) if clinically indicated. If the safety laboratory measurements are performed at a local laboratory, the laboratory normal ranges are provided by the safety laboratory and are filed in the Investigator File. Safety laboratory parameters to be evaluated are:

Hematology:

Red blood cell count, hemoglobin, total and differential White Blood Cell count (WBC), platelet count, Hematocrit, mean corpuscular/cell volume (MCV), mean corpuscular/cellular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW) are routinely performed as part of the complete blood cell count and will be included in the laboratory report.

Serum chemistry:

Total bilirubin, Alkaline Phosphatase (AP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), serum creatinine, sodium, potassium, calcium, CRP.

Pregnancy test:

A β-human choriogonadotropin (β-HCG) pregnancy test will be conducted for all WOCBP at SCR and prior to each vaccination V1 (one-dose vaccination schedule) or V1 and V4 (two-dose vaccination schedule). At SCR a serum β-HCG pregnancy test will be performed; all other pregnancy tests will be conducted as urine β-HCG tests.

Virology:

The following parameters will only be evaluated during the screening period for assessment of inclusion/exclusion criteria:

Human immunodeficiency virus antibody test (anti-HIV)

HBsAG

HCV

Severe Acute Respiratory Syndrome Coronavirus 2 Testing:

SARS-CoV-2 specific antibody test (at SCR only)

SARS-CoV-2 infection test (according to trial schedule)

8.17 Cardiac Assessment

Electrocardiogram (ECG):

A standard 12-lead ECG will be taken at the SCR. At any other visit an ECG is only performed if clinically indicated. For unclear ECGs the investigator can obtain ECG over-read by a local cardiologist.

The investigator will assess the clinical significance for abnormal ECGs. Interpretation support for SCR ECGs is provided in Appendix 3: Interpretation Support for Assessment of SCR ECGs.

8.18 Pregnancy

As per inclusion criteria, WOCBP must have a negative serum pregnancy test at SCR and a negative urine pregnancy test within 24 hours prior to each vaccination. In addition, they must have used an acceptable method of contraception for 30 days prior to the first vaccination, must agree to use an acceptable method of contraception during the trial, and must avoid becoming pregnant for at least 30 days after the last vaccination. Nevertheless, IMP exposed pregnancies cannot be excluded with certainty. Subjects who become pregnant prior to the first vaccination will be excluded from the trial and are regarded as SCR failure. Subjects who become pregnant during the active trial phase (up to and including 1 month [minimum 28 days] after receiving a dose of vaccine) must not receive additional doses of vaccine but may continue other trial procedures at the discretion of the investigator (see Section 4.7). All reports, where the embryo or fetus may have been exposed to the IMP (through maternal exposure) will be followed-up until delivery to collect information on the outcome of the pregnancy (see Section 8.21).

Subjects will be instructed to notify the investigator if it is determined (also after completion of the trial) that they became pregnant either during the trial or within 1 month (minimum 28 days) after receiving the last vaccine dose.

8.19 Reporting

8.20 Reporting of SAE and AESI

All SAEs (collection starts at signing of ICF) and AESIs (collection starts upon receiving first vaccination) occurring throughout the entire course of the trial have to be entered in the eCRF within 24 hours of awareness by the investigators, which triggers an autogenerated output to the Drug Safety (DS)/PV Department of the contract research organization (CRO).

In case the eCRF is unavailable, the SAE and AESI report form is sent via email to the DS/PV Department of the CRO within 24 hours of becoming aware of the SAE or AESI.

Serious adverse events and AESIs should be sent to:

[redacted]

Cc: [redacted]

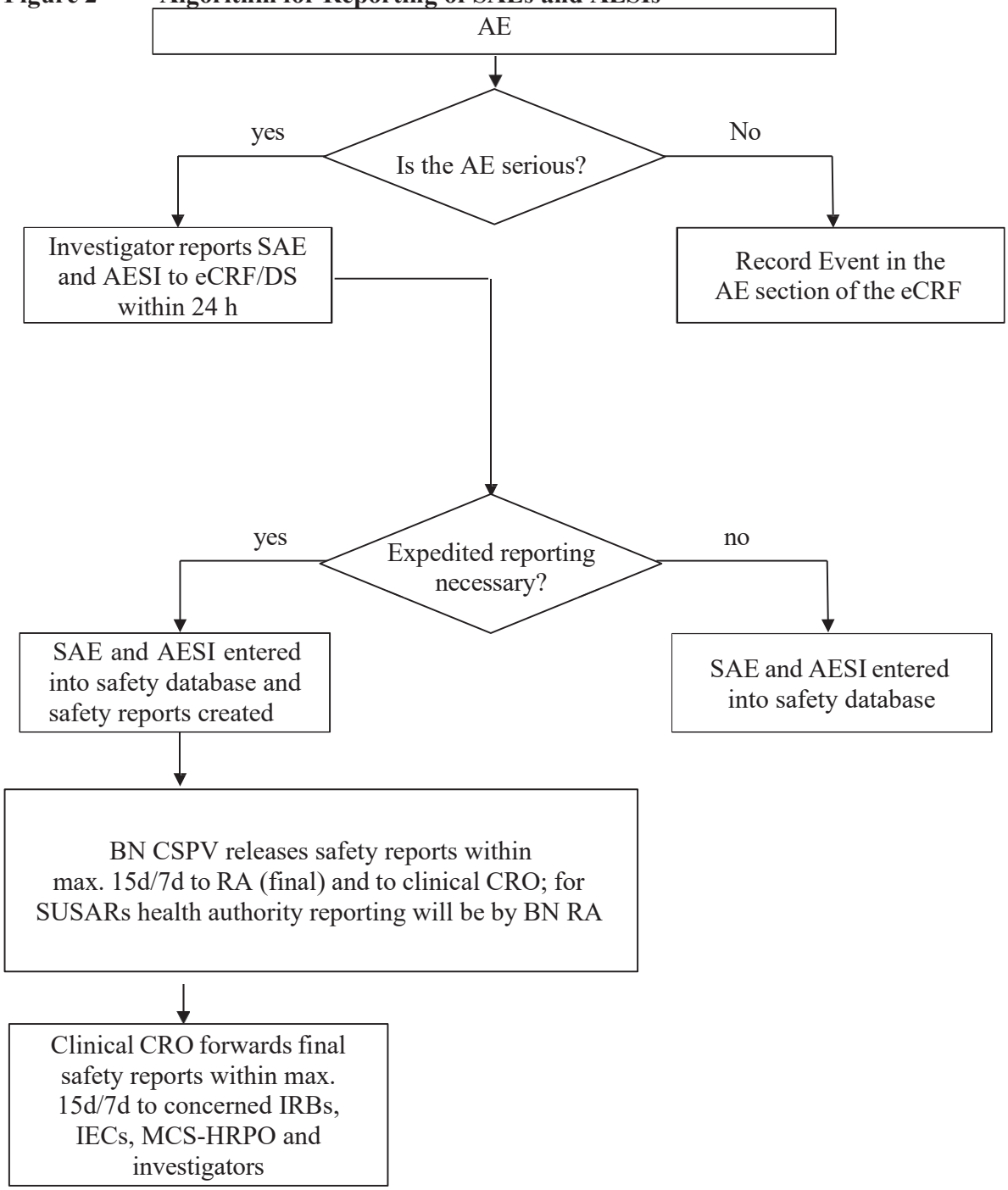
The investigator should not delay reporting because of missing information. Nonetheless, the report should be as complete as possible. This initial notification should include, as a minimum, sufficient information to permit identification of the following:

- the reporter (investigator's name and contact information)
- the subject
- involved trial medication
- AE(s)
- seriousness criterion
- date of onset

BN/CRO is responsible for expedited as well as periodic reporting to the involved regulatory authorities according to applicable laws and guidelines, and for forwarding final safety reports to the clinical CRO.

Regulatory authorities will be notified as soon as possible but no later than 7 days after first knowledge of fatal or life-threatening unexpected SAE with an at least possible relationship to the IMP (serious adverse drug reaction [SADR]) and no later than 15 days after knowledge of any other unexpected SADR. The same process applies for AESIs. The clinical CRO is responsible for reporting to the Ethics Committees or IRBs and HRPO. Figure 2 outlines the reporting process and timelines for SAEs and AESIs.

Figure 2 Algorithm for Reporting of SAEs and AESIs



8.21 Reporting of Pregnancies

If a subject becomes pregnant during the active trial phase (up to and including 1 month [minimum 28 days] after receiving the trial vaccination) this must be recorded on the Pregnancy eCRF (or Pregnancy Notification Report Form if EDC unavailable) within 24 hours of the investigator becoming aware of the event (Section 8.20).

A pregnancy will be followed to term, any premature terminations reported, and the health status of the mother and child including date of delivery and the child's sex and weight will be reported by the investigator to the CRO DS and BN as soon as possible after delivery by recording outcome on the pregnancy eCRF (or Pregnancy Outcome Report Form if EDC unavailable).

Any event during pregnancy fulfilling the criteria for an SAE or AESI will be reported to the CRO DS/PV department (see Section 8.20). However, hospitalization for delivery is a prospectively planned hospitalization and is not considered a SAE, per se.

9 Statistical Considerations

9.1 Randomization Procedure

No randomization procedure will be used for this trial. Recruitment will be performed with a run-in period including enrollment of 6 subjects into Group 1 and 2 (3 subjects in each). Subjects will be vaccinated in a consecutive manner as described in Section 4.1. After completion of V2 and positive review of safety data by the DMC, the DMC will allow for the enrollment of all remaining subjects. Assignment into Group 1 versus Group 2 and 3 will be based on the subjects' baseline SARS-CoV-2 antibody status. For seropositive subjects the reason for seropositivity will be documented: previous COVID-19 infection, mRNA vaccinated, adenovirus-vaccinated, or a mix of those.

Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.

9.2 Sample Size Calculation

Group 1: Up to 30 subjects determined to be seronegative for SARS-CoV-2 antibodies at SCR will be enrolled in Group 1.

Group 2: approximately 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 2.

Group 3: approximately 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 3.

The primary objective of Group 1 is to estimate the level of neutralizing antibodies 2 weeks after the second vaccination. This will be measured by the geometric mean of the neutralizing antibody titers and its 95% confidence interval. Based on published data on the peak neutralizing antibody titers following vaccination with licensed SARS-CoV-2 vaccines, we assume the standard deviation in logarithm 10 (log_{10}) scale will be approximately 0.5. When the sample size is 30, a two-sided 95% confidence interval will extend 0.179 from the observed mean in log_{10} scale. The corresponding 95% confidence interval for the geometric mean will be between 132 and 302 if the point estimate is 200; it will be between 166 and 377 if the point estimate is 250. The lower bound of 132 to 166 is in the similar range of licensed SARS-CoV-2 vaccines (Jin et al., 2021).

The primary objective for Groups 2 and 3 is to estimate the geometric mean of the ratio of neutralizing antibody titers 2 weeks after the single booster vaccination with ABNCoV2 versus the pre-booster level. We assume the same standard deviation of 0.5 in log_{10} scale in both pre-booster and post-booster titers; the correlation coefficient between the pre-booster and post-booster titers is 0.3; and the distribution of the ratio is log-normal. When the sample size is 90, a two-sided 95% confidence interval will extend 0.122 from the observed mean in log_{10} difference. If the observed geometric mean of the ratio is 4 (i.e., a 4-fold increase after booster), the 95% confidence interval will be (3.0, 5.3). When stratified by baseline titer subgroups (<median or ≥median), the lower bound of the 95% confidence interval will be at least 2.7 when the observed ratio is 4 with a subgroup size of 45.

9.3 Multiplicity

Since there is no hypothesis test in this phase 2 trial, multiplicity is not applicable.

9.4 Variables

Please refer to endpoints in Protocol Synopsis Section 1.3.

9.5 Analysis Population

The analysis population includes subjects who meet all of the inclusion and none of the exclusion criteria as defined separately for the three groups in Section 1.3, e.g., subjects assigned to Group 1 must be confirmed seronegative; subjects assigned to Groups 2 and 3 must be confirmed seropositive.

The Safety Analysis Set for all safety variables and population level summaries includes all subjects who received at least 1 dose of the ABNCoV2 vaccine.

The Immunogenicity Analysis Set for immunogenicity variables and population level summaries includes all subjects who are in the Safety Analysis Set and have at least 1 post-vaccination neutralizing antibody titer result. A data review meeting will determine if any protocol violations will affect the immunogenicity outcomes and these subjects will be removed from the analysis set.

9.6 Analysis Methods

9.7 Primary Estimand

9.8 Population Level Summary

Group 1: Geometric mean of SARS-CoV-2 neutralizing antibody titers at 2 weeks after the boost vaccination.

Groups 2 and 3: Geometric mean of the fold-increases, defined as the ratio of SARS-CoV-2 neutralizing antibody titer at 2 weeks divided by the neutralizing antibody titer at baseline (last measurement prior to ABNCoV2 vaccination).

9.9 Intercurrent Events

The following are considered intercurrent events: 1) development of COVID-19 symptoms prior to 2 weeks after the last vaccination (Week 6 in Group 1 and Week 2 in Groups 2 and 3) and having a positive test for SARS-CoV-2 infection, and 2) discontinuation due to AEs from either the second vaccination (Group 1) or the trial. For the primary estimand, the second intercurrent event is not considered as having an impact on the SARS-CoV-2 neutralizing antibody levels and therefore the “treatment policy” strategy will be used. Subjects who come in contact with the virus can have significant interference with the SARS-CoV-2 neutralizing antibody levels and thus will be excluded from the primary analysis. This is equivalent to the “while on treatment” strategy.

9.10 Primary Analysis

The geometric mean of SARS-CoV-2 neutralizing antibody titers (Group 1) and fold increases (Groups 2 and 3) 2 weeks after the last vaccination will be summarized along with their 95% confidence intervals. The arithmetic means and the corresponding 95% confidence intervals on the $\log_{10}$ scale will be calculated using the normal approximation approach. The geometric means and the 95% confidence intervals will be obtained by exponentiating the $\log_{10}$ means and confidence limits. For Groups 2 and 3, analyses of fold-increases will be performed by baseline titer catetories (<median or ≥median) to account for the expected variation in the baseline neutralizing titer due to the different reasons for seropositivity (i.e., previous mRNA vaccination, previous Adenovirus-based vaccination, or previous COVID-19 infection). As the Groups 2 and

3 baseline titer distributions are assumed to be the same, the median baseline neutralizing titer category will be derived from the median of both groups. This will allow for comparability between the geometric fold-increases by dose level (i.e., 100 µg vs. 50 µg).

9.11 Sensitivity and Supportive Analyses

The primary analysis will be supported by descriptive summaries (geometric means, 95% confidence intervals of the geometric means and median) for each time point (pre- and post-vaccination). Response rates, with response being defined as SARS-CoV-2 neutralizing antibody titers above the limit of quantification in Group 1, or having an at least 2-fold or 4-fold increase from baseline in Groups 2 and 3, respectively, will also be summarized and corresponding 95% confidence intervals will be calculated using the Clopper-Pearson method. For these analyses, both the median categories will be used as defined for the primary analysis as well as quartile categories from the pooled Group 2 and 3 baseline neutralizing titer data.

Because the window between vaccination and the primary endpoint measurement is short, the number of subjects with missing data due to the intercurrent event is expected to be small. If there are any, missing data will be imputed using the multiple imputation method as a sensitivity analysis.

The primary analyses will also be performed by age groups as supportive analyses: 18 to <50 years, 50 to <65 years, 65 to <75 years, and ≥75 years.

9.12 Secondary Estimands

9.13 Population Level Summary

Percent of subjects reporting any SAE or AESI assessed as related to trial vaccine within 8 days after vaccination, and percent of subjects reporting any Grade 3 or higher AE assessed as related to trial vaccine within 8 days after vaccination.

9.14 Intercurrent Events

The second intercurrent event is relevant for this estimand. If it is confirmed by the investigator that the subject discontinues the trial within 8 days after vaccination due to AEs, the subject is considered having a related SAE/AESI/Grade 3 or higher AE regardless of the seriousness, severity, or causality of the AE for which the subject discontinued. This is consistent with the "composite" strategy.

9.15 Main Analyses

Percent of subjects reporting any SAE or AESI and percent of subjects reporting any Grade 3 or higher AE assessed as related to trial vaccine within 8 days after any vaccination will be summarized by group and the 95% confidence interval will be calculated using the Clopper-Pearson method.

9.16 Sensitivity and Supportive Analyses

If any subject discontinues the trial within 8 days after vaccination due to AEs, a sensitivity analysis will be performed using the "treatment policy" strategy to handle the intercurrent event, where the subject will not be considered having a related SAE/AESI/Grade 3 or higher AE assessed as related to trial vaccine.

9.17 Exploratory Endpoints

Besides the primary and secondary estimands, the SARS-CoV-2 neutralizing antibody titers will also be assessed at 2 weeks and 4 weeks after prime vaccination, and at 1 week, 4 weeks and 3 months after boost vaccination for Group 1. For Groups 2 and 3, the neutralizing antibody titers will also be assessed at 1 week, 4 weeks, 3 months, 6 months, 1 year and 2 years after vaccination. These data will be summarized descriptively by geometric means and 95% confidence intervals for each time point within each of the three groups. For Groups 2 and 3, analyses will be performed by the baseline neutralizing titer category (<median or ≥median). The data will also be presented by age subgroups.

SARS-CoV-2 neutralizing antibody titers against various variant strains circulating at the time of analysis at 2 weeks after last vaccination for all groups will be summarized similarly.

Total antibody titers measured by ELISA and cellular immune responses measured by ELISPOT will also be summarized by geometric means and their 95% confidence intervals similar to the summaries performed for the neutralizing antibody titers.

The geometric means and 95% confidence intervals will also be plotted as a function of time by group for each of the three groups.

Response rates, with response being defined as SARS-CoV-2 neutralizing antibody titers or total antibody titers above the limit of quantification or having an at least 2-fold increase or 4-fold increase from baseline in Groups 2 and 3, respectively, will also be summarized by time points and groups, and the 95% confidence intervals will be calculated using the Clopper-Pearson method.

9.18 Analyses of Safety

Other safety data will be summarized descriptively. AE data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Solicited and unsolicited AEs will be summarized by system organ class, preferred term, and group. Solicited and unsolicited AEs that meet certain criteria, e.g., ≥ Grade 2, ≥ Grade 3, serious, related to vaccination, within 8 or 29 days after the first or the second vaccination will be summarized similarly.

The proportion of subjects testing positive for COVID-19 infection by timepoint and at any time post vaccination will be summarized by group.

Vital signs and safety laboratory data will focus on subject level changes, thus shifts from baseline will be summarized by group, and graphical presentations may be used.

9.19 Timing of Analyses

9.20 Primary Analysis

In order to inform the phase 3 trial, a primary analysis of immunogenicity and safety will be performed once all subjects have completed the visit for the primary immunogenicity endpoint (2 weeks after the second vaccination for Group 1 or 2 weeks after the single booster vaccination for Groups 2 and 3) or withdrawn early from the trial, and at least the neutralizing antibody testing results are available. Trial data will be cleaned through the primary immunogenicity analysis timepoint, and a data review meeting will be conducted to review the key trial data for the primary analysis and determine the subjects to be excluded from the Immunogenicity Analysis Set.

Reporting will include the analysis of the primary and secondary endpoints (as available), as well as total antibody data and neutralizing antibody titers against various variant strains at 2 weeks after last vaccination. Additionally, demographics and disposition data through the time of analysis will be summarized to describe the population included in the primary analysis. The results of the primary analyses from the primary and secondary endpoints are not expected to change for later reporting. Further details of the summaries to be included in the primary analysis will be included in the SAP.

9.21 Final Trial Analysis

The final analysis for the trial will occur once all subjects have completed the follow up visits or withdrawn early for the trial, and the database has been locked. Analyses of all endpoints will be included at this time for the full clinical study report. Results from the primary analysis is not expected to change, but rather the remaining immunogenicity, safety, and disposition results will

be added to the primary analysis results. Any changes in the data or planned analyses from the primary analysis will be fully documented.

10 Ethical Aspects

10.1 Ethical and Legal Regulations

The PI is to ensure that this clinical trial is conducted in complete accordance with the provisions of the 2013 version of the Declaration of Helsinki, the national laws and other guidelines for the conduct of clinical trials like the ICH GCP to guarantee the greatest possible subject protection.

10.2 Approval by IEC/IRB

The clinical trial protocol must be reviewed by the competent IEC/IRB according to the national laws of the respective CTS before the first subject is included in this trial.

If one of the investigators is a member of one of these committees, he/she may not vote on any aspect of the review of this protocol.

The Sponsor will assure that the IEC/IRB is informed of any amendment to the protocol and any unanticipated problems involving risks to human subjects included in the trial. Such information will be provided to the IEC/IRB at intervals appropriate to the degree of subject risk involved, but not less than once a year. Copies of all correspondence between the investigator and the IEC/IRB must be forwarded immediately to the Sponsor. In case of withdrawal of IEC/IRB approval of the trial, the Sponsor has to be contacted immediately by facsimile, e-mail or telephone.

10.3 Confidentiality and Data Protection

The PI of the respective CTS is obliged to ensure pseudonymity of the subject. He/she has to make sure that all documents including eCRFs provided to third parties (e.g., in the course of a marketing authorization procedure) contain no subject names.

Only a subject and site number may identify subjects. Their name or clinic and subject's medical record number may not be used. The PI keeps separate confidential subject logs for trial recruitment which allows subject numbers to be matched with names and addresses of subjects at any time. Documents not meant to be passed on to third parties have to be stored securely by the PI.

Information collected in the course of the trial may be made available to persons directly involved in this trial (PI and his staff members, monitors, statisticians) or to persons authorized by the Sponsor or to authorities. The Sponsor of the trial will only receive pseudonymized data for analysis.

11 Informed Consent

The ICF and process must comply with ICH GCP guidelines, as well as specific national regulations and/or local laws in the countries where the trial is conducted and must be approved by the appropriate IEC/IRB.

The ICF will document the trial-specific information the Investigator or his/her designee (designee must be listed on the Delegation of Authority log) provides to the subject and the subject's agreement to participate. The Investigator, or designee, must explain in terms understandable to the subject the purpose and nature of the trial, trial procedures, anticipated benefits, potential risks, possible adverse effects, and any discomfort participation in the trial may entail.

Subjects must be informed unequivocally that they may refuse participation in the trial and that they may withdraw from the trial at any time and for whatever reason and that withdrawal of consent will not affect their subsequent medical treatment or relationship with the treating physician.

Subjects also consent to authorize the monitor, quality assurance personnel and regulatory authorities to inspect source documents for data verification and quality assurance purposes. Such verifications will always be conducted at the CTS and under the ethical supervision of the investigator. All aspects of the confidentiality of the subject's data will be guaranteed.

This ICF process must be documented in the subject's source record. Each subject must provide a signed and dated informed consent before any trial-related (nonstandard of care) activities are performed (such as SCR). The initial and any amended signed and dated consent forms must remain in each subject's trial file at the CTS and be available for verification by trial monitor, Sponsor/CRO auditor or competent regulatory authorities at any time. A copy of each signed consent form must be given to the subject at the time that it is signed by the subject.

12 Electronic Case Report Forms and Retention of Records

12.1 Electronic Case Report Forms

Electronic case report forms (eCRFs) will be used to collect the clinical trial data.

All eCRFs are to be filled out completely by authorized CTS personnel. It is the Investigator's responsibility to ensure that all subject data entered in the eCRF (including discontinuations or changes in trial vaccine or other medications) are accurate, complete, legible and supported by the subject's medical records. The Investigator attests to this by providing electronic signature within the EDC system.

The eCRFs for any subject leaving the trial should be completed at the time of the final visit or shortly thereafter. For subjects not fulfilling the eligibility criteria the minimum information documented in the eCRF is the ICF information, demographics, and reason for screen failure.

The eCRFs exists within an electronic data capture (EDC) system with controlled access managed by BN or its authorized representative for this trial. Trial staff will be appropriately trained in the use of eCRFs and application of electronic signatures before the start of the trial and before being given access to the EDC system. Original data and any changes of data will be recorded using the EDC system, with all changes tracked by the system and recorded in an electronic audit trail. After database lock, the Investigator will receive a copy of the subject data (e.g., paper, CD-ROM or other appropriate media) for archiving at the CTS.

12.2 Retention of Records

The Investigator/trial staff must maintain adequate and accurate records to enable the conduct of the trial to be fully documented and the trial data to be subsequently verified. All essential documents, as listed in ICH GCP guidelines, will be retained by the Investigator for at least 2 years after the date the last marketing application is approved for the drug in the indication being investigated and until there are no pending or contemplated marketing applications; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after formal discontinuation of clinical development of the drug.

The Investigator must notify and obtain written approval from BN before destroying any clinical trial documents or images (e.g., scan, radiograph, ECG tracing) at any time. The Sponsor will inform the Investigator of the date that the trial records may be destroyed or returned to BN.

Should an Investigator wish to assign trial records to another party, advance written notice must be given to the Sponsor. Bavarian Nordic must also be notified in advance and provide express written approval of any change in the maintenance of clinical trial documents, should the Investigator choose to move trial records to another location.

If the Investigator cannot guarantee the aforementioned archiving requirements at the CTS for all such documents, special arrangements must be made between the Investigator and BN to store these documents in secure sealed containers away from the CTS. These documents must be able to be returned in their secure sealed containers to the CTS for auditing purposes.

13 Monitoring of the Trial

Contract research organization (contact information to be found in the “Responsibilities” section in the beginning of this protocol) will be contracted to perform monitoring services according to ICH GCP. Monitoring will be conducted according to the monitoring plan which must be approved by BN. The monitoring plan will outline the monitoring strategy (including rationale)

and will specify in detail the items for source data verification and other tasks, to be performed by the CRA.

Monitoring will be conducted through onsite visits with the Investigator and site staff, remote monitoring, as well as any appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the trial is conducted in compliance with the protocol, SOPs, and other written instructions and regulatory guidelines, and to ensure the quality and integrity of the data.

To assure the accuracy of data collected in the eCRFs, it is mandatory that the monitor have direct access to all original source documents, including all electronic medical records at reasonable times and upon reasonable notice. During the review of source documents, every effort will be made to maintain the privacy and confidentiality of all subjects during this clinical trial.

The site needs to maintain records to identify the nature and location of all source documents as well as essential documents.

13.1 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be either on the part of the participant, the investigator, or the trial site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The PI or designee will be responsible for identifying and recording all deviations which are defined as isolated occurrences involving a procedure that did not follow the protocol or a protocol-specific procedure. All deviations from the protocol and actions taken will be recorded in the source data and placed in the trial specific regulatory file. Protocol deviations must be sent to the local IEC/IRB per their guidelines. The site PI/trial staff is responsible for knowing and adhering to their IEC/IRB requirements. Further details about the handling of protocol deviations will be included in a trial specific procedure.

14 Audits and Inspections

Site audits may be carried out by the BN quality assurance department or designee at any time during or after completion of this trial. All documents pertinent to the trial must be made available to the designated auditor. Subject privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or designee.

In addition, representatives from local, state, or federal regulatory authorities may choose to inspect a trial site at any time before, during, or after completion of the clinical trial. The

Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection. In the event of such an inspection, BN will be available to assist in the preparation. All pertinent trial data should be made available as requested by the Regulatory Authority for verification, audit, or inspection purposes.

15 Responsibility of the Investigator

The Investigator should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC, or the regulatory authority(ies).

The PI agrees to carry out the trial in accordance with the guidelines and procedures outlined in this clinical trial protocol. The PI especially consents to strictly adhere to the ethical aspects of this protocol (see Section 10).

Changes to the protocol require written “Amendments to the protocol” and written approval by the IEC/IRB and the PI. Changes are allowed only if the trial value is not reduced and if they are ethically justifiable. If warranted, the subject information has to be changed accordingly.

It is within the responsibility of the investigator that the eCRF is completed in a timely manner after each subject visit and electronically signed after the subject has completed the trial for each subject participating in the trial.

At the conclusion of the trial, the investigator will return all partially used, unused and empty drug containers to the assigned warehouse, a vendor selected by BN or the drug containers will be destroyed at the CTS according to local legal requirements.

The investigator may ask to terminate participation in the trial due to administrative or other reasons. If this should be the case, appropriate measures which safeguard the interests of the participating subjects must be taken after verification and consultation with the PI.

The investigator will maintain appropriate medical and research records for this trial, in compliance with the ICH E6 Guideline for GCP and regulatory and institutional requirements for the protection of confidentiality of subjects. He/she will permit authorized representatives of the Sponsor and regulatory authorities to review (and, when required by applicable law, to copy) clinical records for the purposes of quality reviews, audits/inspections, and evaluation of the trial safety and progress.

The PI agrees to follow the detailed publication policy included in the clinical trial agreement.

By signing this protocol, the PI confirms that he/she has read the entire clinical trial protocol, agrees to its procedures, and will comply strictly with the formulated guidelines.

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17 Appendices

Appendix 1: Toxicity Scale for Laboratory Values

Grade 1 or Grade 2 toxicity is only graded according to "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA, 2007)" if the value is outside of the institutional normal range applicable for this trial.

Estimating severity grade
For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

Grade 1	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with daily activities.
Grade 2	An AE which is sufficiently discomforting to interfere with daily activities, but does not require medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose).
Grade 3	An AE which prevents daily activities and which requires medical intervention (non-narcotic pain reliever or other nonprescription medication are not considered "medical intervention" for this purpose).
Grade 4	Life-threatening or disabling

Serious or life-threatening AEs
ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a Grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: Seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

Appendix 2: Grading Scale for Lymphadenopathy

A grading scale for lymphadenopathy would apply as follows:

Grade 0 (normal finding):	No palpable lymph nodes or lymph nodes up to a diameter of 1 cm, soft, non-tender
Grade 1 (mild):	Slightly palpable lymph nodes or lymph nodes up to a diameter of 1 cm, bilaterally enlarged lymph nodes, signs of tenderness
Grade 2 (moderate):	Markedly palpable lymph nodes or lymph node diameter exceeds 1 cm, bilaterally enlarged lymph nodes, pain, skin redness, warmth, limiting instrumental daily life activities
Grade 3 (severe):	Markedly palpable lymph nodes or lymph node diameter exceeds 2 cm, generalized enlargement of lymph nodes, severe pain, general symptoms like fever and sweating limiting self-care daily activities

Appendix 3: Interpretation Support for Assessment of Screening ECGs

For a clearer and mutual understanding of inclusion criterion #8, the following provides clarifying explanations and examples pertaining to eligibility for enrollment.

Examples of subjects **eligible for enrollment**:

- Non-specific ST and T wave changes are not considered clinically significant and subject can be enrolled.
- Sinus bradycardia which does not require clinical intervention is not considered clinically significant and subject can be enrolled.
- Subjects who present with atrial disease which do not require clinical intervention, e.g., a pacemaker or drug treatment are allowed to be enrolled, as these can be considered not clinically significant. Examples are premature atrial contractions or ectopic atrial beats.
- Occasional premature ventricular contractions which do not require clinical intervention are not considered clinically significant and subject can be enrolled.
- First degree AV block or PR interval prolongations are also acceptable as long as they do not require clinical intervention, i.e., do not represent an indication for a pacemaker, and therefore the condition can be classified as not clinically significant.
- Right or left axis deviation which does not require clinical intervention is not considered clinically significant and subject can be enrolled.
- QTc prolongations <500 ms which do not require clinical intervention are not considered clinically significant and subject can be enrolled. QTc prolongations ≥500 ms which do not require clinical intervention should be discussed with the Medical Monitor before enrollment.

Examples of subjects **NOT eligible for enrollment**:

- Second or third degree atrioventricular block could represent significant heart disease and subject should not be enrolled.
- Incomplete left bundle branch blocks could represent significant heart disease and subject should not be enrolled.
- Significant ventricular disease represented by complete intraventricular conduction defects (complete left or right bundle branch block) must be considered clinically significant and subjects presenting with any such condition should not be enrolled. Left anterior or posterior intraventricular fascicular blocks or hemiblock could represent ventricular disease and subject should not be enrolled.
- ST elevation consistent with ischemia, subject should not be enrolled.
- Two premature ventricular contractions in a row, subject should not be enrolled.

Appendix 4: Signature Page

Investigator Signature Page

Herewith I agree that I have read and fully understand this protocol:

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

This protocol describes necessary information to conduct the trial. I agree that I will conduct the trial according to the instructions given within this protocol. Furthermore, I agree that I will conduct this trial according to International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP), the 2013 version of the Declaration of Helsinki, as well as applicable local legal and regulatory requirements in the respective countries. Additionally, I will follow all applicable national regulations requirements (e.g., German Arzneimittelgesetz). I agree that all information revealed in this protocol is handled strictly confidentially.

Additionally, I will permit trial related monitoring, audits, Institutional Review Board (IRB) / Independent Ethics Committee (IEC) review and regulatory inspections, providing direct access to source data/documents.

(Date) (Signature) Name, MD

Principal Investigator (PI)

Address

Coordinating Investigator Signature Page

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

I agree that the protocol was written according to international ethical and scientific quality standards (ICH-GCP), in compliance with the 2013 version of the Declaration of Helsinki and local legal and regulatory requirements applicable in the respective country.

17-Oct-2021 | 10:03 CEST

[redacted]

(Date) (Signature) [redacted]

Principal Investigator (PI)

[redacted]

Sponsor Signature Page

By signing the protocol:

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

the undersigned parties agree that the protocol was written according to international ethical and scientific quality standards (ICH-GCP), in compliance with the 2013 version of the Declaration of Helsinki and local legal and regulatory requirements applicable in the respective country.

15-Oct-2021 | 15:53 CEST

Coordinating Author
and Medical Monitor

[redacted]

(Date) (Signature) [redacted]

Bavarian Nordic GmbH

15-Oct-2021 | 15:29 CEST

Biostatistician

[redacted]

(Date) (Signature) [redacted]

Bavarian Nordic Inc.

Appendix 5: Summary of Changes for Amendment#1 to the Clinical Trial Protocol

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

Date of Edition 2.0: 1-Jul-2021

Rationale

The protocol Edition 2.0 has been created to implement and incorporate revisions requested by the Paul Ehrlich Institute (PEI) on 22-Jun-2021.

Changes

Changes/ added terms are highlighted in **bold** letters in the text (table below), deleted terms are marked using ~~strikethrough~~.

Table 4 Description

Clinical Trial Protocol Edition 1.0, dated 29-Apr-2021 Previously written:	Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Changed to:
1.2 Definitions, page 8	**1.2 Definitions, page 8**
	Completed vaccination regimen with an authorized SARS-COV2 vaccine. “Authorized” SARS-CoV2 vaccine refers to authorization status at SCR, i.e., subjects can be eligible if they previously received investigational vaccines that have since been authorized for emergency use or granted full market licensure. “Completed” means administration of all vaccinations as described in the respective label. **Added to explain definition used in inclusion criteria**
1.3 Protocol Synopsis	**1.3 Protocol Synopsis**

Clinical Trial Protocol Edition 1.0, dated 29-Apr-2021 Previously written:	Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Changed to:
Exploratory Objectives page 11 To assess SARS-CoV-2 specific peak humoral immune responses after the prime-boost regimen (Group 1) or the booster dose (Group 2) of the ABNCoV2 vaccine and compare these peak immune responses to those of subjects who had a previous SARS-CO-V-2 vaccination less than 3 months before SCR:.	**Exploratory Objectives page 11** To assess SARS-CoV-2 specific peak humoral immune responses after the prime-boost regimen (Group 1) or the booster dose (Group 2) of the ABNCoV2 vaccine ~~and compare these peak immune responses to those of subjects who had a previous SARS-CO-V-2 vaccination less than 3 months before SCR:.~~ **Exploratory objective adapted**
Trial Design page 12 … In a run in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) will be vaccinated at 1 clinical trial site in a consecutive manner before opening up to full enrollment in Group 1 and 2. … The trial will begin with a run-in phase. This phase will include 6 adults (3 subjects in each of Groups 1 and 2, respectively) who will be vaccinated at 1 clinical trial site in a consecutive manner before opening the trial to full enrollment.	**Trial Design page 12** …In a run in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) will be vaccinated at 1 clinical trial site in a consecutive manner, **with an at least 48 hours interval between the first and second subject of each group, then the second and third subject dosed on consecutive days**, before opening up to full enrollment in Group 1 and 2… The trial will begin with a run-in phase. This phase will include 6 adults (3 subjects in each of Groups 1 and 2, respectively) who will be vaccinated at 1 clinical trial site in a consecutive manner **(at least 48 hours between first and second subject in each group, and 1 subject per 1 day for second to third subject in each group)** before opening the trial to full enrollment. **Specified to address comments from PEI**

Clinical Trial Protocol Edition 1.0, dated 29-Apr-2021 Previously written:	Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Changed to:
Inclusion Criteria Page 13 2. Seropositive (Group 2): Previous COVID-19 disease or previous vaccination with an authorized SARS-CoV-2 vaccine at least 3 months before SCR, and a positive qualitative test for SARS-CoV-2 antibodies at SCR. 6. Female subjects of childbearing potential (WOCBP) and male subjects who are sexually active with a WOCBP must agree to the use of an effective method of birth control from at least 30 days prior to administration of the vaccine until 30 days after the vaccination. 15. Recent blood donation (including platelets, plasma and red blood cells) within 4 weeks prior to SCR, or planned blood donations at any time during the trial.	**Inclusion Criteria Page 13** 2. Seropositive (Group 2): Previous COVID-19 disease or **previously completed** vaccination **regimen** with an authorized SARS-CoV-2 vaccine at least 3 months before SCR, and a positive qualitative test for SARS-CoV-2 antibodies at SCR. 6. Female subjects of childbearing potential (WOCBP) ~~and male subjects who are sexually active with a WOCBP~~ must agree to the use of an effective method of birth control from at least 30 days prior to administration of the vaccine until 30 days after the vaccination. **Male subjects who are sexually active with a WOCBP must agree to the use of an effective method of birth control from the day of administration of the vaccine until 30 days after the vaccination.** 15. Recent blood donation (including platelets, plasma and red blood cells) within 4 weeks prior to SCR, or planned blood donations ~~at any time~~ during **the active** trial **phase.** **Adapted to address comments from PEI**
1.5 Responsibilities Page	**1.5 Responsibilities Page**
[redacted]	[redacted] **Office number replaced by mobile number**
4.1 Experimental Design	**4.1 Experimental Design**

Clinical Trial Protocol Edition 1.0, dated 29-Apr-2021 Previously written:	Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Changed to:
4.2.2 Active Trial Phase Page 28 In total, 210 subjects will be enrolled in this trial. Recruitment will start with a run-in phase of 6 subjects (3 in each of Groups 1 and 2, respectively, dosed on consecutive days) before opening up enrollment to both treatment groups. **…** After a positive DMC recommendation, enrollment of the rest of Groups 1 and 2 of the trial will commence.	**4.2.2 Active Trial Phase Page 28** In total, 210 subjects will be enrolled in this trial. Recruitment will start with a run-in phase of 6 subjects (3 in each of Groups 1 and 2, respectively, **at least 48 hours interval between the first and second subject in each group, the second and third subject dosed** on consecutive days) before opening up enrollment to both treatment groups. … After a positive DMC recommendation **for each of the groups**, enrollment of the rest of Groups 1 and 2 of the trial will commence. **Addressing PEI comments on dosing of consecutive days**
… The first 6 subjects (3 subjects each in Group 1 and Group 2) will be vaccinated consecutively, at a maximum 1 per day.	… ~~The first 6 subjects (3 subjects each in Group 1 and Group 2) will be vaccinated consecutively, at a maximum 1 per day.~~ **Deleted as specified in sections before**
4.4 Data Monitoring Committee	**4.4 Data Monitoring Committee**
The primary responsibility of the DMC is to review and evaluate the accumulated trial safety data after the run in phase and make recommendations to proceed to open enrollment for remaining subjects. If an event occurs which fulfills the trial halting rules (see Section 4.10 for further details) after the run in phase, the DMC will…	The primary responsibility of the DMC is to review and evaluate the accumulated trial safety data ~~after the run in phase~~ and make recommendations to proceed to open enrollment for remaining subjects. If an event occurs which fulfills the trial halting rules (see Section 4.10 for further details) ~~after the run in phase~~, the DMC will… **Addressing PEI comments on dosing of consecutive days and DMC involvement**
9.1 Randomization Procedure	**9.1 Randomization Procedure**

Clinical Trial Protocol Edition 1.0, dated 29-Apr-2021 Previously written:	Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Changed to:
Subjects will be vaccinated in a consecutive manner (max 1 subject/day).	Subjects will be vaccinated in a consecutive manner ~~(max 1 subject/day)~~ as described in Section 4.1 **Addressing PEI comments on dosing of consecutive days**
9.5 Analysis Population Page 50	**9.5 Analysis Population Page 50**
A blinded data review meeting will determine if any protocol violations will affect the immunogenicity outcomes and these subjects will be removed from the analysis set.	A ~~blinded~~ data review meeting will determine if any protocol violations will affect the immunogenicity outcomes and these subjects will be removed from the analysis set. **Typo removed**
	9.7 Timing of Analysis page 9.7.1 Primary Analysis 9.7.2 Final Trial Analysis **Chapter added to specify procedure of analysis e.g. addition of interim analysis**

Appendix 6: Summary of Changes for Amendment #2 to the Clinical Trial Protocol

An Open Label Phase 2 Trial to Evaluate the Safety, Tolerability and Immunogenicity of the ABNCoV2 Vaccine in SARS-CoV-2 Seronegative and Seropositive Adult Subjects

Date of Edition 3.0: 15-Oct-2021

Rationale

The protocol Edition 3.0 has been created to implement the addition of a further treatment group of seropositive subjects (Group 3) receiving a dose of 50µg of IMP as well as adapting sample sizes of Group1 and 2.

In addition, clarification of the inclusion and exclusion criteria were implemented, reflecting the current real-world experience in Germany, where many subjects have either mix&match regimens or a previous COVI-19 disease plus a single vaccine administration as reason for seropositivity. Due to the variety of reasons for seropositivity, stratification will therefore be now done based on baseline neutralizing antibody titer, instead of initial regimen.

The new 50 µg dose group was selected based on preliminary results from the ongoing Phase 1 first-in-human trial, indicating a dose saturation between 25µg and 70 µg dose groups, in terms of neutralizing antibody responses. For further details on Phase 1 data, please refer to the updated Investigator's Brochure, Section 5.

Changes

Changes/ added terms are highlighted in **bold** letters in the text (table below), deleted terms are marked using ~~strikethrough~~.

Table 5 Description

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
1.1 Abbreviation	**1.1 Abbreviation**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
	cVLP capsid virus-like particle added PBS Phosphate buffered saline
1.2 Definitions	**1.2 Definitions**
Active Trial Phase: The period from the first vaccination up to and including 1 month (28-35 days) after receiving the last trial vaccination. For Group 1 this includes V1-V7. For Groups 2, this includes V1-V4.	**Active Trial Phase:** The period from the first vaccination up to and including 1 month (28-35 days) after receiving the last trial vaccination. For Group 1 this includes V1-V7. For Groups 2 **and 3**, this includes V1-V4.
Completed vaccination regimen …… "Completed" means administration of all vaccinations as described in the respective label.	Completed vaccination regimen … "Completed" ~~means administration of all vaccinations as described in the respective label~~ **includes full primary vaccination as described in the labeling of the initial vaccine, but also includes any mix/match series of 2 doses any authorized COVID-19 vaccine.**
Last Vaccination: For Group 1 (seronegative) the last vaccination is the second vaccination of their two-dose prime-boost regimen, scheduled to be received at V4. For Groups 2 (seropositive) the last vaccination is the single boost vaccination received at V1.	**Last Vaccination:** For Group 1 (seronegative) the last vaccination is the second vaccination of their two-dose prime-boost regimen, scheduled to be received at V4. For Groups 2 **and 3** (seropositive) the last vaccination is the single boost vaccination received at V1.
1.3 Protocol Synopsis	**1.3 Protocol Synopsis**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
No of sites and countries 1 to 3 sites, Germany and/or Netherlands **Vaccination Dose and Schedule** Each subject will receive either 2 doses of 100 µg (Group 1), 1 dose of 100 µg (Group 2) ABNCoV2 vaccine. **Trial Duration** Approximately 104 weeks for initially seropositive subjects receiving 1 vaccination (Group 2). **Sample Size** A total of 210 healthy adult subjects will be enrolled into 2 groups: 60 subjects determined to be seronegative for SARS-CoV-2 antibodies at screening (SCR) will be enrolled in Group 1. 150 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 2. Enrollment will be stratified by reason for seropositivity, i.e., previous COVID-19 disease or type of previous vaccination received (mRNA vs. Adenovirus-based vaccination) such that at least 40 subjects and up to 70 subjects are enrolled in each stratification group (previous COVID-19 disease, previous mRNA vaccination or previous Adenovirus-based vaccination).	**No of sites and countries** ~~1 to 3~~ **2** sites, Germany ~~and/or Netherlands~~ **Vaccination Dose and Schedule** Each subject will receive either 2 doses of 100 µg (Group 1), 1 dose of 100 µg (Group 2) or **1 dose of 50 µg (Group 3)** ABNCoV2 vaccine. **Trial Duration** **Up tp** 104 weeks for initially seropositive subjects receiving 1 vaccination (Group 2 and **Group 3**). **Sample Size** A total of 210 healthy adult subjects will be enrolled into 2 groups: Up to 30 subjects determined to be seronegative for SARS-CoV-2 antibodies at screening (SCR) will be enrolled in Group 1. ~~Approx. 90~~ Up To **150** subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 2 **(100 µg)**. ~~Enrollment will be stratified by reason for seropositivity, i.e., previous COVID-19 disease or type of previous vaccination received (mRNA vs. Adenovirus-based vaccination) such that at least 40 subjects and up to 70 subjects are enrolled in each stratification group (previous COVID-19 disease, previous mRNA vaccination or previous Adenovirus-based vaccination).~~ **Approx. 90 subjects determined to be seropositive for SARS-CoV-2 antibodies at SCR will be enrolled in Group 3 (50 µg).**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
Exploratory Endpoints Groups 2 (seropositive subjects): SARS-CoV-2 neutralizing antibody titers at 1 week, 4 weeks, 3 months, 6 months, 1 year and 2 years after vaccination. … Endpoints for each group will be analyzed separately. Groups 1 and 2 (all subjects): Cellular immune response, e.g., by Interferon-γ / Interleukin-4 ELISPOT, at 1 week after the last vaccination, i.e., after the second vaccination in initially seronegative subjects and after the single boost vaccination in initially seropositive subjects.	**Exploratory Endpoints** Groups 2 **and 3** (seropositive subjects): SARS-CoV-2 neutralizing antibody titers at 1 week, 4 weeks, 3 months, 6 months, 1 year and 2 years after vaccination. … ~~Endpoints for each group will be analyzed separately.~~ Groups 1, 2 **and 3** (all subjects): Cellular immune response, e.g., by Interferon-γ / Interleukin-4 ELISPOT, at 1 week after the last vaccination, i.e., after the second vaccination in initially seronegative subjects and after the single boost vaccination in initially seropositive subjects.
Trial Design	**Trial Design**

A phase 1, dose ranging trial in healthy, seronegative adults is currently ongoing to evaluate safety and reactogenicity with doses up to 70µg

In a run in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) …

This phase 2 trial … compared to a single boost vaccination with 100 µg ABNCoV2 in initially seropositive subjects, …vaccination or previous COVID-19 disease (Group 2) at least 3 months prior to enrollment (Table 1).

Table 1 Treatment Groups and Number of Subjects

Dose (/0.3 mL)	Treatment Groups		Total N
	Group 1 Seronegative	Group 2 Seropositive	
100 µg	60 subjects[a]	150 subjects[a]	210

N = number of subjects
[a] n = 3 of Group 1 and Group 2 (total 6 subjects) will be enrolled in the run-in phase before opening the trial to full enrollment.

For the seropositive subjects (Group 2), enrollment will be stratified by reason for seropositivity: previous COVID-19 disease, previous mRNA-based vaccination regimen, or previous Adenovirus-based vaccination regimen. Enrollment will occur in such a way

A prior phase 1, dose ranging trial in healthy, seronegative adults ~~is currently ongoing to~~ evaluates safety and reactogenicity with doses up to 70µg

For this Phase 2 trial, in a run in phase 6 adults (comprising of 3 subjects in each Group 1 and 2) .....**Group 3 subjects will be enrolled after completion of Group 2 enrollment.**

This phase 2 trial … compared to a single boost vaccination of 100 µg **(Group 2) or 50 µg (Group 3)** of ABNCoV2 in initially seropositive subjects, … vaccination or previous COVID-19 disease at least ~~3 months~~ **90 days** prior to enrollment (Table 1).

Table 1 Treatment Groups and Number of Subjects

Dose (/0.3 mL)	Treatment Groups	Total N	
	Seronegative	Seropositive	
100 µg	Group 1 **30 subjects[a]**	Group 2 **90 subjects[a]**	**120**
50 µg		**Group 3 90 subjects**	**90**
Total		210	

N = number of subjects
[a] n = 3 of Group 1 and Group 2 (total 6 subjects) will be enrolled in the run-in phase before opening the trial to full enrollment.

For the seropositive subjects (Groups 2 **and 3**), ~~enrollment will be stratified by reason for~~ **the reason for seropositivity will be documented** (previous COVID-19 disease, previous mRNA-based vaccination regimen,

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
that at least 40 and up to 70 subjects will be included in each stratification group. The trial will begin with a run-in phase. … … After a positive DMC recommendation, enrollment of the rest of Groups 1 and 2 of the trial will commence.	**or previous Adenovirus-based vaccination regimen**). ~~Enrollment will occur in such a way that at least 40 and up to 70 subjects will be included in each stratification group.~~ **Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.** ~~The trial will begin with a run-in phase. … After a positive DMC recommendation, enrollment of the rest of Groups 1 and 2 of the trial will commence.~~
Inclusion Criteria	**Inclusion Criteria**
#2 …Seropositive (Group 2): Previous COVID-19 disease or previously completed vaccination regimen with an authorized SARS-CoV-2 vaccine at least 3 months before SCR, and a positive qualitative test for SARS-CoV-2 antibodies at SCR. …	#2: …Seropositive (Group 2 **and Group 3**): Previous COVID-19 disease or previously completed vaccination regimen with an authorized SARS-CoV-2 vaccine at **least 90** days before ~~SCR~~ **planned trial vaccination**, and a positive qualitative test for SARS-CoV-2 antibodies at SCR. …**Receipt of a single dose of an authorized COVID-19 vaccine regimen in subjects with a previous diagnosis of COVID-19 or a mix/match series of 2 doses of any authorized COVID-19 vaccine will be considered as a completed vaccination.**
Exclusion Criteria	**Exclusion Criteria**
	#1 Groups 2 and 3 only: History of COVID-19 infection and subsequent receipt of more than one licensed or candidate SARS-CoV-2 vaccine.
1.5 Responsibilities	**1.5 Responsibilities**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
	
Trial Schedule	**Trial Schedule**
AE/SAE/AESI recording	AE/SAE/AESI recording **starting at screening visit** **AESIs are not collected until vaccine has been received at visit 1.**
2.4 Clinical Trial Data with ABNCoV2 Vaccine	**2.4 Clinical Trial Data with ABNCoV2 Vaccine**
Preliminary safety results in 18 subjects dosed up to 25 µg did not show any relevant safety findings. No final results from this trial are available yet. For further dose justification, please refer to Section 2.3	Preliminary safety results in **45** subjects dosed up to **70 µg** did not show any relevant safety findings. No final results from this trial are available yet. For further dose justification, please refer to Section 2.3
2.5 Rationale	**2.5 Rationale**
This trial will, therefore, evaluate both an initial two-dose vaccination regimen of ABNCoV2 in seronegative subjects as well as a single dose boost regimens of ABNCoV2 in seropositive subjects.	This trial will, therefore, evaluate ~~both~~ an initial two-dose vaccination regimen of ABNCoV2 in seronegative subjects as well as ~~a~~ **two** single dose boost regimens of ABNCoV2 in seropositive subjects.
2.7.1 Potential Risk	**2.7.1 Potential Risk**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
As with all vaccines, … site pain, erythema, swelling, pruritus or induration, as well as systemic inflammatory reactions including unspecific flu-like symptoms such as fever, headache, nausea, muscle pain, chills or fatigue.	**Based on available data from COUGH-1 study, there have been no SAEs reported**. As with all vaccines, …site pain **and/or tenderness,** erythema, swelling, pruritus or induration. **The above mentioned were reported most commonly in the COUGH-1 study following both doses of study vaccine.** Also, systemic inflammatory reactions including flu-like symptoms such as fever, headache, nausea, muscle pain, chills or fatigue **can occur**. **Headache, chills and fatigue were reported most commonly in the COUGH-1 study after first dose of study vaccine. After the second dose of study vaccine, myalgia was mostly commonly reported, followed by nausea, hyperhidrosis and a general feeling of warmth without fever**.
4.1 Experimental Design	**4.1 Experimental Design**
The trial will evaluate a homologous prime-boost regimen with 100 µg ABNCoV2 in … single boost vaccination with 100 µg ABNCoV2 in initially seropositive subjects (Group 2), as defined by a positive qualitative test for SARS-CoV-2 antibodies and a history of SARS-CoV-2 vaccination or previous COVID-19 disease at least 3 months prior to enrollment (Table 1).	The trial will evaluate a homologous prime-boost regimen with 100 µg ABNCoV2 in … single boost vaccination with either 100 µg **or 50 µg dose** of ABNCoV2 in initially seropositive subjects (Groups 2 **and 3**), as defined by a positive qualitative test for SARS-CoV-2 antibodies and a history of SARS-CoV-2 vaccination or previous COVID-19 disease at **least 90 days** prior to enrollment (Table 1). … **Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
2.7.1 Potential Risk	**2.7.1 Potential Risk**
As with all vaccines, there is a risk of an allergic reaction or an anaphylactic event. Trial site staff will observe subjects for at least 30 minutes after each vaccination and, in the event that a severe allergic reaction and/or dyspnoea might occur, appropriate medical treatment and supervision will be readily available. As with all vaccines, there is also a risk of temporary mild to moderate injection site reactions, such as injection site pain, erythema, swelling, pruritus or induration, as well as systemic inflammatory reactions including unspecific flu-like symptoms such as fever, headache, nausea, muscle pain, chills or fatigue	**Based on available data from COUGH-1 study, there have been no SAEs reported.** As with all vaccines, there is a risk of **temporary mild to moderate injection site reactions, such as injection site pain and/or tenderness, erythema, swelling, pruritus or induration. The above mentioned were reported most commonly in the COUGH-1 study following both doses of study vaccine. Also,** systemic inflammatory reactions including flu-like symptoms such as fever, headache, nausea, muscle pain, chills or fatigue can occur**.** Headache, chills and fatigue were reported most commonly in the COUGH-1 study after first dose of study vaccine. **After the second dose of study vaccine, myalgia was mostly commonly reported, followed by nausea**, **hyperhidrosis and a general feeling of warmth without fever**
4.1 Experimental Design	**4.1 Experimental Design**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
…with 100 µg ABNCoV2 in initially seropositive subjects (Group 2), as defined by a positive qualitative test for SARS-CoV-2 antibodies and a history of SARS-CoV-2 vaccination or previous COVID-19 disease at least 3 months prior to enrollment …In total, 210 subjects will be enrolled in this trial.	… with **either** 100 µg **or 50 µg dose** of ABNCoV2 in initially seropositive subjects (Groups 2 **and 3**), as defined by a positive qualitative test for SARS-CoV-2 antibodies and a history of SARS-CoV-2 vaccination or previous COVID-19 disease at least ~~3 months~~ **90 days** prior to enrollment …In total, **approximately** 210 subjects will be enrolled in this trial. **Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.**
4.2.2 Active Trial Phase	**4.2.2 Active Trial Phase**
At V1/Day 1 each subject in Groups 1 and 2 will receive 1 dose of 100 µg ABNCoV2 vaccine administered IM into the deltoid muscle of the upper arm (preferably the non-dominant arm). … For Group 1, the memory aid will be collected for the 8-day period for both vaccinations.	At V1/Day 1 each subject in Groups 1 and 2 will receive 1 dose of 100 µg ABNCoV2 vaccine, and **Group 3 will receive 1 dose of 50 µg ABNCoV2 vaccine** administered IM into the deltoid muscle of the upper arm (preferably the non-dominant arm). … For **each subject** in Group 1, **two** memory aids will be collected ~~for the 8-day period~~ (**one for each** of the two vaccinations).
4.2.5 Early Discontinuation	**4.2.5 Early Discontinuation**
	Subject's receipt of an approved booster vaccination for SARS-CoV-2.

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
6.1 Production, Packaging and Labelling	**6.1 Production, Packaging and Labelling**
	For dilution buffer phosphate buffered saline (PBS) is used and released by Bavarian-Nordic A/S, DK. The packages and vials of dilution buffer are labeled according to regulatory requirements.
6.2. Shipment, Storage and Handling	**6.2. Shipment, Storage and Handling**
Details on shipment, storage and handling of the IMP are provided in the pharmacy manual.	Details on shipment, storage and handling of the IMP (**and comparable information for the diluent to be used for Group 3**) are provided in the pharmacy manual.
7.3 Future Use of Lab Specimen	**7.3 Future Use of Lab Specimen**
… data collected from subjects vaccinated with competitor vaccines. Further, remaining samples might be used for assay development and controls. Subjects will be asked to consent to storage/future use of samples and will be informed about data protection measures.	data collected from subjects vaccinated with ~~competitor~~ **other COVID-19** vaccines. Further, remaining samples might be used for assay development and controls. Subjects will be **invited** to consent to storage/future use of samples and will be informed about data protection measures.
8.1.2.3 AEs and SAEs	**8.1.2.3 AEs and SAEs**
AESIs are reportable by the clinical investigators as per SAE protocol provisions.	~~AESIs are reportable by the clinical investigators as per SAE protocol provisions.~~ **All AEs are collected from signing of ICF, however should not be considered by investigator to be an AESI unless occurring since receipt of first vaccination**.

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:
8.1.3 Relevant Medical History	**8.1.3 Relevant Medical History**
	In particular, subjects with prior COVID-19 infection need to include the infection and dates in the medical history section of the eCRF.
8.1.4 Prior and Concomitant Medication	**8.1.4 Prior and Concomitant Medication**
	In addition, subjects with previous SARS-CoV-2 vaccination will include the specific vaccine received by dose and date in the eCRF, regardless of the time elapsed prior to SCR.
8.1.5 Prohibited Medication	**8.1.5 Prohibited Medication**
	In case official guidelines require booster vaccinations to maintain valid COVID-19 vaccination certificates, approved booster vaccinations for SARS-CoV-2 are allowed for Groups 2 and 3 within the follow-up period, however subjects will be withdrawn from further follow up.
8.1.11 Cardiac Assessment	**8.1.11 Cardiac Assessment**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
	A standard 12-lead ECG will be taken at the SCR. At any other visit an ECG is only performed if clinically indicated. For unclear ECGs the investigator can obtain ECG over-read by a local cardiologist. ~~All further clinically indicated ECGs will be rated by a cardiologist who would also receive the baseline ECG for review. The workflow communication will be provided in a separate manual.~~
8.2.1 Reporting of SAE and AESIs	**8.2.1 Reporting of SAE and AESIs**
All SAEs and AESIs occurring throughout the entire course of the trial …	All SAEs (**collection starts at signing of ICF**) and AESIs (**collection starts upon receiving first vaccination**) occurring throughout the entire course of the trial …
8.2.2 Reporting of Pregnancies	**8.2.2 Reporting of Pregnancies**
… must be on the Pregnancy Notification Report Form within 24 hours ... A pregnancy will be followed to term, ...on a Pregnancy Outcome Report Form.	... must be recorded on the Pregnancy **eCRF (or Pregnancy** Notification Report Form **if EDC unavailable**) within 24 hours ... A pregnancy will be followed to term, ...by recording outcome on the pregnancy **eCRF (or Pregnancy** Outcome Report Form **if EDC unavailable).**
9.1 Randomization Procedure	**9.1 Randomization Procedure**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
After completion of V2 and positive review of safety data by the DMC, the DMC will allow for the enrollment of all remaining subjects in both treatment groups. Assignment into Group 1 versus Group 2 will be based on the subjects' baseline SARS-CoV-2 antibody status. Group 2 will be stratified by seropositive category: previous COVID-19 infection, mRNA vaccinated, or adenovirus-vaccinated.	After completion of V2 and positive review of safety data by the DMC, the DMC will allow for the enrollment of all remaining subjects ~~in both treatment groups~~. Assignment into Group 1 versus Group 2 **and 3** will be based on the subjects' baseline SARS-CoV-2 antibody status. **For seropositive subjects the reason for seropositivity will be documented**: previous COVID-19 infection, mRNA vaccinated, adenovirus-vaccinated, **or a mix of those**. **Due to the timing of the addition of Group 3, enrollment into Group 2 will be completed prior to enrolling subjects into Group 3. Therefore, no randomization will be required for the seropositive subjects.**
9.2 Sample Size Calculation	**9.2 Sample Size Calculation**
	Whole chapter was adapted to reflect addition of Group 3 and adapting size of different treatment groups
9.6.1.3 Primary Analysis	**9.6.1.3 Primary Analysis**

Clinical Trial Protocol Edition 2.0, dated 1-Jul-2021 Previously written:	**Clinical Trial Protocol Edition 3.0, dated 15-Oct-2021 Changed to:**
	For Groups 2 and 3, analyses of fold-increases will be performed by baseline titer catetories (<median or ≥median) to account for the expected variation in the baseline neutralizing titer due to the different reasons for seropositivity (i.e., previous mRNA vaccination, previous Adenovirus-based vaccination, or previous COVID-19 infection). As the Groups 2 and 3 baseline titer distributions are assumed to be the same, the median baseline neutralizing titer category will be derived from the median of both groups. This will allow for comparability between the geometric fold-increases by dose level (i.e., 100 µg vs. 50 µg).
9.6.3 Exploratory Endpoints	**9.6.3 Exploratory Endpoints**
These data will be summarized descriptively by geometric means and 95% confidence intervals for each time point and each group (Group 1 and Group 2). Response rates, ... having an at least 2-fold increase or 4-fold increase from baseline in Groups 2 and 3, respectively, will also be summarized by time points and groups, …	These data will be summarized descriptively by geometric means and 95% confidence intervals for each time point **within each of the three groups**. **For Groups 2 and 3, analyses will be performed by the baseline neutralizing titer category (<median or ≥median).** Response rates, ... having an at least 2-fold increase **or 4-fold increase** from baseline in Groups 2 **and 3, respectively**, will also be summarized by time points and groups...