CLINICAL TRIAL PROTOCOL

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)

Version 1.6

Date: 31 March 2021

Confidentiality Statement

PROTOCOL TITLE 'First-in-human trial of the Coronavirus virus-like particle subunit vaccine ABNCoV2 in SARS-CoV-2-naïve adult volunteers in good health'

Protocol ID	**COUGH-1**
Short title	Safety and tolerability of ABNCoV2
EudraCT number	2020-004621-22
Version	1.6
Date	31 March 2021
Protocol authors	Benjamin Mordmüller, MD Jessica Brosnahan Meral Esen, MD Diane Egger-Adam, PhD Matthew McCall, MD, PhD Merel J. Smit, MD Morten Agertoug Nielsen, PhD
Principal investigator(s)	**Benjamin Mordmüller, MD** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3619499 Email: benjamin.mordmueller@radboudumc.nl
Coordinating investigator	**Matthew McCall, MD, PhD** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3615363 Email: matthew.mccall@radboudumc.nl
Clinical investigator(s)	**Merel J. Smit, MD** Radboudumc, Department of Medical Microbiology Tel: +31 (0)24 3619515 Email: merel.smit@radboudumc.nl
Sponsor	**Stichting Radboud universitair medisch centrum** **Department of Medical Microbiology** **Geert Grooteplein-Zuid 10**

	6525 GA, Nijmegen, The Netherlands **Sponsor representative:** **Heiman Wertheim, MD, PhD** Clinical Microbiology Laboratory Tel: +31 (0)24 3614281 Email: Heiman.Wertheim@radboudumc.nl
Subsidising party	European Union Horizon 2020 research and innovation action (RIA) project 101003608, PREVENT-nCoV
Independent expert (s)	**Arjan van Laarhoven, MD, PhD** Radboudumc Department of Internal Medicine Tel: +31 (0)24 361 69 80. Email: Arjan.vanLaarhoven@radboudumc.nl
Laboratory sites	**Eric Aaldring** Radboudumc Clinical Chemical Laboratory Tel: +31 (0)24 3616997 Email: Eric.Aaldring@radboudumc.nl **Heiman Wertheim, MD, PhD** Clinical Microbiology Laboratory Tel: +31 (0)24 3614281 Email: Heiman.Wertheim@radboudumc.nl
Pharmacy	**Rob ter Heine, PharmD, PhD** Radboudumc Clinical Pharmacy – Clinical Trial Unit Tel: +31 (0)24 3616405 Email: R.terHeine@radboudumc.nl
Antibody Assays	**Morten Nielsen, PhD** University of Copenhagen Faculty of Health Science Institute for Immunology and Microbiology Centre for Medical Parasitology Tel: +45 35326803 Email: mortenn@sund.ku.dk

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Principal Investigator: Benjamin Mordmüller, MD Department of Medical Microbiology		**31 March 2021**

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AEFI	**Adverse Event Following Immunization**
ALT	**Alanine aminotransferase**
AR	**Adverse Reaction**
AS	**Adjuvant System**
AST	**Aspartate aminotransferase (AST)**
AV	**AdaptVac**
BEVS	**Baculovirus Expression Vector System**
BMI	**Body Mass Index**
BP	**Bloodpressure**
CA	**Competent Authority**
CBC	**Complete blood count**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CDT	**Carbohydrate-deficient transferrin**
cGMP	**current Good Manufacturing Practice**
CoV	**Coronavirus**
COVID-19	**Coronavirus disease 2019**
CRF	**Case Report Form**
CRO	**Contract Research Organization**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**

ELISA	**Enzyme-Linked immunosorbent assay**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FDA	**Food and Drug Administration**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
GLP	**Good Laboratory Practice**
GMP	**Good Manufacturing Practice**
HBV	**Hepatitis B virus**
HCV	**Hepatitis C virus**
HIV	**Human immunodeficiency virus**
HPV	**Human papillomavirus**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ID	**Identifier**
IEC	**Independent Ethics Committee**
IgG	**Immunoglobulin G**
IM	**Intramuscular**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
IRB	**Institutional Review Board**
ITT	**Intention-To-Treat**
IU	**International Unit**
LSM	**Local safety monitor**
MERS	**Middle East respiratory syndrome**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**

NFU	**Nederlandse Federatie van Universitair Medische Centra**
NHP	**Non-human primate**
NPC	**Non-pharmaceutical interventions**
NZW	**New Zealand White**
PBMC	**Peripheral Blood Mononuclear Cell**
PCR	**Polymerase chain reaction**
PI	**Principal investigator**
PP	**Per protocol**
RBC	**Red blood cell**
RBD	**Receptor Binding Domain**
RNA	**Ribonucleic Acid**
RTCCS	**Radboudumc Technology Center Clinical Studies**
SAE	**Serious Adverse Event**
SARS	**Severe Acute Respiratory Syndrome**
SARS-CoV	**Severe Acute Respiratory Syndrome Coronavirus**
SARS-CoV-2	**Severe Acute Respiratory Syndrome Coronavirus 2**
SEM	**Standard error of the mean**
SOP	**Standard Operating Procedure**
SMC	**Safety Monitoring Committee**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
S-Protein	**SARS-CoV-2 Spike Protein**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**

TLR	**Toll Like Receptor**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
VE	**Vaccine efficacy**
VLP	**Virus Like Particle**
cVLP	**Capsid Virus Like Particle**
WHO	**World Health Organization**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a zoonotic virus, primarily causing respiratory symptoms in humans, ranging from very mild to life threatening. The current outbreak of SARS-CoV-2 was first reported in late 2019 and has spread rapidly around the world, leading the World Health Organization (WHO) to declare a pandemic. A vaccine could complement non-pharmaceutical interventions (NPC), in order to protect vulnerable populations by reducing virus-spread, decrease the load on health care systems and reduce the social and economic impact of NPC. The ABNCoV2 vaccine is intended to protect against coronavirus disease 2019 (COVID-19) and limit spread of SARS-CoV-2.

Objective: The main objectives of the trial are to assess the safety and tolerability of two doses of ABNCoV2, formulated with and without the adjuvant MF59, in healthy adult volunteers and to identify the dosage and formulation that optimizes the immunogenicity-tolerability ratio 14 days following first vaccination with ABNCoV2.

Study design: COUGH-1 is a phase 1, single centre, open labelled trial in healthy, adult, SARS-CoV-2-naïve volunteers. The trial involves first-in-human administration, pre-defined, sequential dose escalation of ABNCoV2, and adjuvant selection. It intends to inform dosage and formulation for subsequent clinical development.

Study population: Healthy, SARS-CoV-2-naïve, adult female and male volunteers, 18-55 years old.

Intervention: ABNCoV2 is a virus-like particle vaccine. It will be administered as two intramuscular injections in groups of up to 9 volunteers. The pre-defined escalation schedule will start with 6 µg ABNCoV2, followed by 12, 25 and 50 µg with a maximum dose of 70 µg. MF59-adjuvanted and non-adjuvanted formulations will be tested in parallel to detect superiority or futility of the MF59-adjuvanted against the non-adjuvanted formulation at the 6, 12 and 25 µg dosage. Approval for further dose escalation and choice of adjuvant use will be provided by a safety monitoring committee (SMC), supported by pre-defined analyses of safety, tolerability and immunogenicity data at day 14 post-first-vaccination. Recruitment for the two best (safe, tolerable and most immunogenic) regimens will continue until 12 volunteers per regimen have been immunized.

Main study parameters/endpoints: Safety – number of at least possibly related Grade 3 adverse events (AE) and serious adverse events (SAE) from time of first administration of ABNCoV2 until the end of the follow-up period and number and severity of at least possibly related solicited AEs within one week following administration of ABNCoV2. Immunogenicity – concentration of ABNCoV2-specific antibodies 14 days following first vaccination.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: There is no direct benefit from participation in this trial. Information about their own general health status may be a potential indirect benefit for participants. It will be made clear that the vaccine is experimental and may not protect against COVID-19. Participating in this trial includes risks associated with intramuscular ABNCoV2 administration, immune-response against the vaccine and blood sampling. Volunteers will experience frequent follow-up visits, physical examinations, screening for HIV, hepatitis B and hepatitis C, a pregnancy test (for females) and COVID-19 diagnostics.

1. INTRODUCTION AND RATIONALE

1.1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in late 2019 after a number of pneumonia cases with unknown cause were reported in Wuhan City, China. Coronavirus disease 2019 (COVID-19), the disease caused by SARS-CoV-2, spread rapidly through China and other countries worldwide. In March 2020, the World Health Organisation (WHO) declared a global pandemic [1].

SARS-CoV-2 is a Betacoronavirus (CoV), causing respiratory and systemic symptoms in humans, ranging from very mild to life threatening. Coronaviruses are a family of viruses that usually cause mild to moderate upper-respiratory tract illnesses in humans. Four human coronaviruses (HCov-229E, HCov-HKU1, HCov-NL63 and HCov-OC43) are endemic, causing disease with a peak in winter months [2]. However, in the past two decades three new coronaviruses have emerged from animal reservoirs and have caused serious illness, complications and death. SARS coronavirus (SARS-CoV) emerged in November 2002 and caused severe acute respiratory syndrome (SARS), until it disappeared in 2004 [3, 4]. Transmitted from an animal reservoir in camels, the Middle East respiratory syndrome (MERS) caused by the MERS coronavirus (MERS-CoV) was identified in 2012 [5]. MERS continues to cause sporadic and localized outbreaks. The third coronavirus to emerge in this century is SARS-CoV-2. SARS-CoV-2 is a zoonotic virus, likely originating from bats and transmitted to humans via an intermediary mammalian host.

Whilst the genome of all three novel CoVs is similar, the S gene of SARS-CoV-2 is divergent to the other CoVs and information about protective immune responses to the virus is scarce, so far. All CoVs cause primarily respiratory illnesses – from common cold to severe respiratory distress, but other complication may occur, particularly with SARS-CoV-2 infections.

The SARS-CoV-2 virus is transmitted through respiratory droplets, with a mean incubation period of 4-6 days. Most patients display symptoms within 14 days of infection [6]. The most prominent symptoms are fever, cough, and fatigue. However, a number of other symptoms have been identified as being associated with COVID-19, including loss of smell and taste, sore throat, diarrhoea, fatigue, neurological symptoms, cardiac symptoms, coagulopathies, and renal damage amongst others [7-9].

Many patients experience only mild symptoms, and some are asymptomatic, leading to increased transmission due to social contact whilst feeling well. COVID-19 is associated with significant mortality in at-risk groups. The main risk factor for complications and death is old age, typically associated with a number of pre-existing conditions. Geographic variations may also exist, although different testing strategies bias mortality estimates.

Although a worldwide concerted effort was made to contain the virus by way of non-pharmaceutical interventions (NPC) such as local “lockdowns”, social contact restrictions, travel restrictions and contact tracing with resultant quarantine, it continues to run rampant in countries such as the US and the UK. In the Netherlands and other European countries incidence decreased over the summer but since September infections resurge, and incidence remains high (in March 2021 ~30,000 new cases/week in the Netherlands) despite re-implementation of NPCs. As of March 2021, there had been nearly 120 million cases world-wide, with more than 2.5 million deaths [10].

The ABNCoV2 vaccine is a sub-unit vaccine using a non-infectious virus-like particle (VLP) platform to optimize immunogenicity. A stabilized version of the receptor binding domain (RBD) of SARS-CoV-2 Spike glycoprotein (S) is bound to an VLP, and through vaccination is presented to the immune system in order to evoke a response resulting in protection from infection. Most vaccine candidates targeting SARS-CoV-2 and other members of the family *Coronaviridae* consist of S antigens and are optimized to achieve high invasion inhibition activities. Proof-of-concept studies of S antigen-based vaccine candidates have shown high-level vaccine efficacy and transmission-blocking activity in dromedaries and other *Camelidae* [11-13], the main host of the Middle East respiratory syndrome coronavirus (MERS).

Besides ABNCoV2, more than 200 vaccines against SARS-CoV-2 are under development [14]. First candidates have reached Phase 3 clinical trials and results have been published in the form of press releases [15-17], two providing more detail in form of an interim analyses [18, 19]. Three studies have reached approximately 100 cases, which allows a reasonably estimate of a clinically relevant vaccine efficacy (VE). Observed VE was 60-90%. All three trials used candidates based on the S antigen as the immunogen and have observed significant immunogenicity following two vaccinations [20-22]. Two use an RNA-vaccine-platform and one a recombinant adenovirus. Follow up times are still short (median of ~3 months) but vaccination campaigns based on emergency-use authorization have already started in some countries. Platform technologies that enable such a fast development (RNA, viral vector) are relatively new and have only been used in the framework of clinical trials – many without an infectious disease prevention indication. RNA vaccines have mostly been used as proof-of-concept vaccines for cancer. In the few studies against infectious diseases, they have shown satisfactory but not particularly long-lasting or broad responses [23, 24]. The Oxford/AstraZeneca platform has been used extensively but without success in malaria and tuberculosis vaccine development. In addition, systemic reactogenicity was significant in all three studies.

Virus like particles (VLPs) that display antigen on their surface are a technology to increase immunogenicity of soluble protein subunit vaccines and have an extensive safety record; e.g. as human papilloma virus (HPV) and hepatis B vaccines. Besides induction of better immune responses, memory and specificity of the response may also be improved. Since VLPs cannot replicate they can be safely used in immunocompromised and elderly populations, two populations at particular risk for severe COVID-19. In COUGH-1 a novel VLP technology that allows covalent, directional, high density binding of different proteins on the VLP surface will be used. Besides the immunological advantages, this decreases development time significantly, although not to levels as fast as RNA vaccines because proteins must still be expressed. The VLP itself consists of the coat protein of AP205, a structural protein of a phage (a virus infecting bacteria) that does not infect eukaryotes (including humans). AP205 VLPs have never been used in humans but the closely related Qβ phage-based VLP system has been tested in clinical trials [25-27].

In pre-clinical models ABNCoV2 immunogenicity has been superior to the other platform technologies. In addition, it is optimized to comply with the WHO target product profile for SARS-CoV-2 vaccines [28]. If successful, it will be developed as a second-generation SARS-CoV-2 vaccine.

1.2 Rationale

There is currently no cure for COVID-19. The disease itself, as well as the essential measures put in place to reduce its spread, are wreaking havoc on communities, economies and health systems worldwide. Not only are economies suffering as a result of lockdowns and restrictions, with recessions imminent, but healthcare systems are overwhelmed, resulting in increased morbidity and mortality due to other diseases and routine immunization programmes are being disrupted leaving millions of children worldwide at risk of preventable diseases. An effective vaccine is essential and urgently required, in order to protect populations all over the world from COVID-19. Even though first vaccines have been registered, the need for new products remains high. Since COVID-19 is a worldwide pandemic, no single supplier will be able to produce enough vaccine to cover the world's population and not all vaccines in the pipeline are effective, safe and easy to deploy. A larger portfolio of vaccines will also increase resilience of the public health response including faster reaction to control spread of new virus strains and potential vaccine-escape mutants. In addition, the target product profile will differ between populations as will vaccine safety and acceptability. Hence, the use of different technologies will improve safety, coverage, effectivity, efficiency [29] and together with NPCs, control of the epidemic. The ABNCoV2 sub-unit vaccine also has the potential to prevent COVID-19 in health care workers, as well as limit spread of the disease in epidemic settings.

2. OBJECTIVES

2.1 Primary Objectives

Safety:

- To assess safety and tolerability of two doses of ABNCoV2, formulated with and without MF59 in healthy adult volunteers.

Immunogenicity:

- To identify a dosage that optimizes the tolerability-immunogenicity ratio 14 days following first vaccination with ABNCoV2.

3. STUDY DESIGN

This first-in-human phase 1 trial of ABNCoV2 is a single center, sequential dose-escalation, open labelled trial to establish the safety and tolerability of two doses of ABNCoV2, formulated with and without MF59 in healthy, adult, SARS-CoV-2-naïve volunteers. The immunological objective of this trial is to identify a dosage that optimizes the immunogenicity-tolerability ratio 14 days following first vaccination with ABNCoV2. The trial will be carried out by the Radboud University Medical Center (Radboudumc).

The trial involves first-in-human administration, dose escalation of ABNCoV2 and adjuvant selection. Volunteers will be screened for eligibility and receive two vaccinations by intramuscular injection. While we cannot predict with certainty the safety in human subjects, we have adopted a safety-orientated staggered trial design with ascending doses of ABNCoV2. Seven groups of volunteers (n=6) will receive a given dose of ABNCoV2, either with or without MF59, followed by a booster with the same dose and formulation four weeks after the first vaccination. All vaccinations will be given as intramuscular injection. The pre-defined escalation schedule will start with 6 µg ABNCoV2, with a maximum dose of 70 µg. Dose-escalation will proceed only in absence of protocol-defined safety signals. MF59-adjuvanted and non-adjuvanted formulations will be tested in parallel at the first three escalation steps (Group 1-3) to detect superiority or futility of the MF59-adjuvanted against the non-adjuvanted formulation. Group 1A (n=3) will receive two doses of 6 µg non-adjuvanted ABNCoV2, Group 1B (n=3) will receive two doses of 6 µg adjuvanted ABNCoV2, Group 2A (n=3) will receive two doses of 12 µg non-adjuvanted ABNCoV2, Group 2B (n=3) will receive two doses of 12 µg adjuvanted ABNCoV2, Group 3A (n=3) will receive two doses of 25 µg non-adjuvanted ABNCoV2 and Group 3B (n=3) will receive two doses of 25 µg adjuvanted ABNCoV2. After the first vaccinations in group 3 subjects, a decision will be taken as to whether to proceed with or without adjuvant in the subsequent groups. Group 4 (n=6) will receive two doses of 50 µg adjuvanted or non-adjuvanted ABNCoV2, Group 5 (n=6) will

receive two doses of 70 µg adjuvanted or non-adjuvanted ABNCoV2, Group 6 (n=6-9)[1] will receive the optimal or second-highest adjuvanted or non-adjuvanted ABNCoV2 dose, and Group 7 (n=6-9) will receive the highest adjuvanted or non-adjuvanted ABNCoV2 dose achieved (Table 1). Up to forty-two (n=42) subjects will be enrolled, as well as one reserve subject per group.

ABNCoV2 administration will be staggered at each dose-escalation and addition of adjuvant such that the first subject in each group will be administered ABNCoV2 and observed for the occurrence of any AEs. The second subject in each dose group will not receive their dose of ABNCoV2 sooner than 6 days after the first subject has received their first dose of ABNCoV2 (Figure 1). Escalation to the next higher dosage group will be dependent upon no safety signals arising as described below and will follow the same staggered algorithm as shown in Figure 1.

Decision on further dose escalation and adjuvant use will be made by the investigator and sponsor and will be discussed and approved by a safety monitoring committee (SMC), supported by pre-defined analysis (see below) of safety, tolerability and immunogenicity data up to day 14 post-vaccination. The SMC will review the safety data of Group 1-3 up to day 14 after each participant's respective first vaccination before proceeding to Group 4. The data to be reviewed includes the safety data (solicited AEs through day 7 and unsolicited AEs through day 14 post ABNCoV2 administration with and without adjuvant; see below) and clinically significant laboratory tests collected at 24 hours and 7 days following first ABNCoV2 administration for all subjects in the specified dose group.

The ABNCoV2 dose will be escalated until:

- The maximal dose of 70 µg is reached, *or*
- The vaccine is too reactogenic and/or not tolerated, *or*
- Immunogenicity-dose response curve is saturated (no further increase or decrease in antibody concentration with increasing dose)

The highest safe/tolerable and the next lower dosage will be re-tested (in groups 7 and 6, respectively) to increase power to detect adverse reactions at the optimal dose and to inform dosing studies in the elderly population. If the immunogenicity dose-response is saturated *before* reaching a safety/tolerability limit, then this dose and the next lower dose will be re-

[1] Group 6 and 7 ensure that at least 12 volunteers have received the highest and second-highest dosage. In case that dose-escalation is completed before 50 µg is reached, n=9 volunteers will be recruited, otherwise n=6 (Figure 1 for examples).

tested. At completion of the trial, 12 volunteers will have received the highest and second-highest dosage.

Table 1. Study groups

Study group	Number of subjects	ABNCoV2 dosage	MF59-adjuvant	Administration Route
Group 1A	3	6 µg	no	intramuscular
Group 1B	3	6 µg	yes	intramuscular
Group 2A	3	12 µg	no	intramuscular
Group 2B	3	12 µg	yes	intramuscular
Group 3A	3	25 µg	no	intramuscular
Group 3B	3	25 µg	yes	intramuscular
Group 4	6	50 µg	t.b.d.*	intramuscular
Group 5	6	70 µg	t.b.d.*	intramuscular
Group 6	6	Second-highest/optimal dose**	t.b.d.*	intramuscular
Group 7	6	Highest dose**	t.b.d.*	intramuscular

* Whether Groups 4-7 will receive a MF59-adjuvanted formulation is dependent on the superiority or futility of the MF59-adjuvanted against the non-adjuvanted formulation in Groups 1-3.

** In principle, the highest and second-highest safe/tolerable doses will be re-tested to increase power to detect adverse reactions at the optimal dose and to inform dosing studies in the elderly population. If the immunogenicity-dose response is saturated before reaching a safety/tolerability limit, then the highest achieved dose and the next lower dosage will be re-tested.

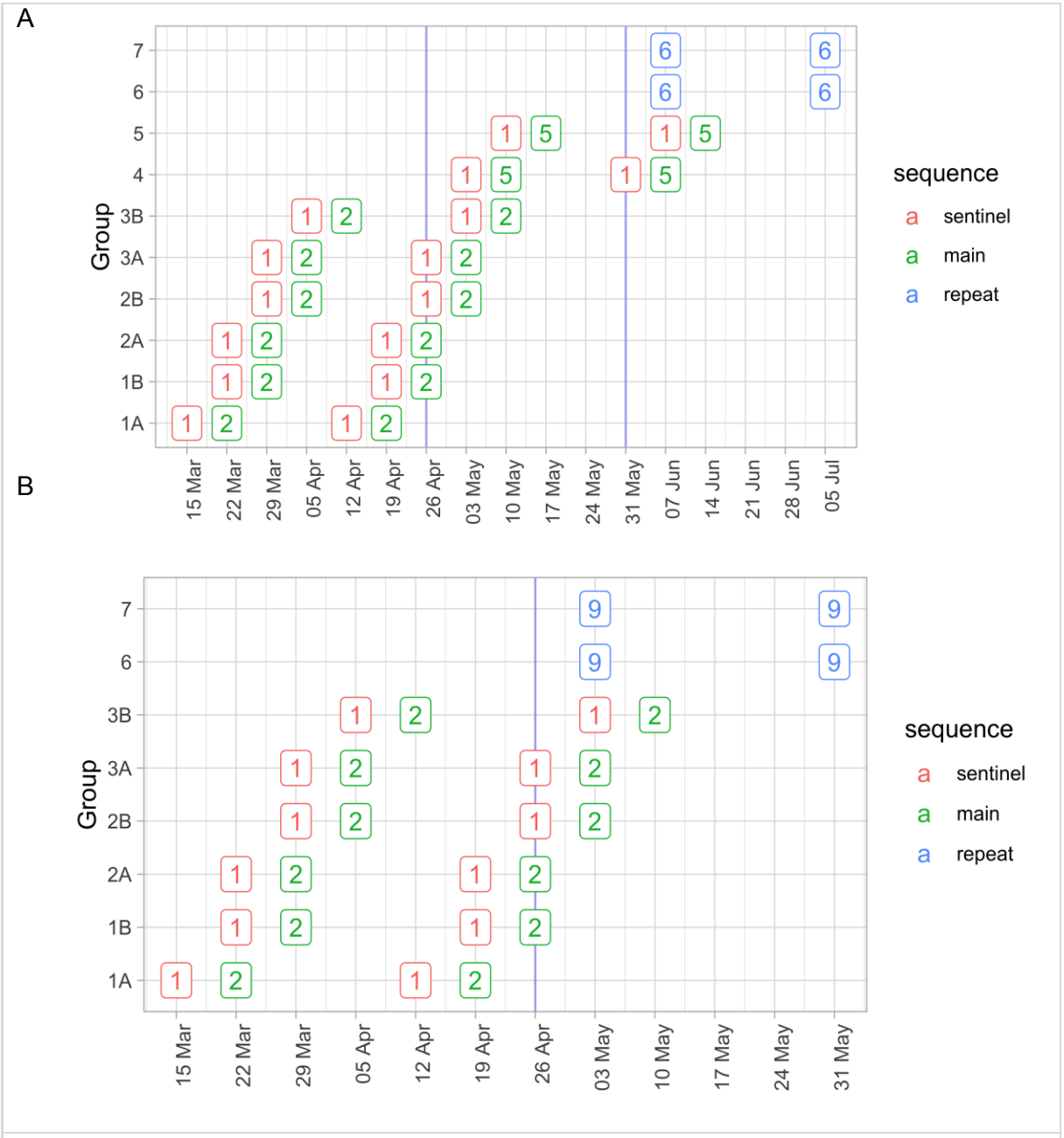

Figure 1. Staggered dosing trial design for ABNCoV2 administration. A) Example algorithm in case that escalation up to 70 µg (Group 5) is required; B) example algorithm for an escalation up to 25 µg (Group 3). For both examples the date of first vaccination is arbitrarily set at 15 March 2021; coloured numbers indicate n, the vertical blue lines are the decision points for further use of adjuvant and dosage in groups 6 and 7, respectively (in example B both decisions will be made at the same time)

3.1 Visit intervals

The permissible study visit intervals are indicated in Table 2.

Table 2. Permissible study visit intervals

Visit	Type of visit	Day of visit	Allowed interval
Visit 1	Screening	I1-28 to I1-1	- 28 to -1 days
Visit 2	Inclusion	I1-3	± 2 days
Visit 3	Administration 1st ABNCoV2 dose	I1	± 1 day
Visit 4	Follow-up	I1+1	0 day
Visit 5	Follow-up	I1+2	0 day
Visit 6	Follow-up	I1+7	± 1 day
Visit 7	Follow-up	I1+14	± 1 day
Visit 8	Inclusion	I2-3	± 2 days
Visit 9	Administration 2nd ABNCoV2 dose	I2	± 1 day
Visit 10	Follow-up	I2+1	0 day
Visit 11	Follow-up	I2+2	0 day
Visit 12	Follow-up	I2+7	± 1 day
Visit 13	Follow-up	I2+14	± 1 day
Visit 14	Follow-up	I2+42	± 2 days
Visit 15	Follow-up	I2+91	± 2 days
Visit 16	Follow-up (end-of-study visit)	I2+168	± 3 days

4. STUDY POPULATION

4.1 Population

The study population will be comprised of adult male and female healthy subjects aged 18-55 at time of first ABNCoV2 administration. A maximum of 42 subjects will be enrolled to participate in the study, in addition to up to 7 reserve subjects (1 reserve subject per dosing group). The investigator will ensure that all subjects being considered for the study meet the eligibility criteria described in section 4.2 and 4.3. Subject eligibility is to be established and confirmed by checking all inclusion/exclusion criteria at both screening and inclusion (baseline). A relevant record of the eligibility criteria will be stored with the source documentation at the study site.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Subject must sign written informed consent to participate in the trial.
2. Subject is able to understand planned study procedures and demonstrate

comprehension of the protocol procedures and knowledge of the study by passing a quiz (assessment of understanding). Subjects must score at least 80% correct on a multiple-choice quiz. If they do not score 80% on the initial quiz, the protocol information will be reviewed with them, and they will have the opportunity to retest.

3. In the opinion of the investigator, the subject can and will comply with the requirements of the protocol.
4. Subjects are available to attend all study visits and are reachable by phone throughout the entire study period from day -1 until 24 weeks following last vaccination (end of study).
5. Subject is a male or non-pregnant and non-lactating female age ≥ 18 and ≤ 55 years and in good health at time of ABNCoV2 administration.
6. Subject agrees to their general practitioner (GP) being informed about participation in the study and agrees to sign a form to request the release by their GP, and medical specialist when necessary, of any relevant medical information concerning possible contra-indications for participation in the study to the investigator(s).
7. The subject agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period according to current Sanquin guidelines.
8. Female subjects of non-childbearing potential may be enrolled in the study. Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause. All other female subjects must agree to use continuous adequate contraception[2] for the duration of the study. Female subjects must have a negative pregnancy test at the inclusion visit.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Any clinically significant abnormal finding on clinical examination or laboratory screening tests according to the US Food and Drug Administration (FDA) Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventative Vaccine Clinical Trials [30].
2. History of COVID-19 infection.
3. Chronic use of immunosuppressive drugs or other immune modifying drugs within six

[2] Acceptable forms of female contraception include: established use of oral, injected or implanted hormonal contraceptives; intrauterine device or intrauterine system; barrier methods (condoms or diaphragm with additional spermicide); male partner's sterilization (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate); true abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptom-thermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Adequate contraception does not apply to subjects of childbearing potential with partners of the same sex.

months prior to study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period.
4. Positive urine toxicology test for cannabis, cocaine or amphetamines at inclusion.
5. Screening tests positive for SARS-CoV-2, SARS-CoV-2 antibodies, Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV), or Hepatitis C Virus (HCV).
6. Receipt of any investigational or non-registered product (drug or vaccine) other than the study product in the 30 days preceding enrolment or during the study period.
7. Participation in any other clinical study in the 30 days prior to the start of the study or during the study period.
8. Immunization with any vaccines within the past four weeks or planned receipt of a vaccine during the study period with the exception of a licensed SARS-CoV-2 vaccine, given within the framework of the national SARS-CoV-2 vaccination campaign. The time between last vaccination with ABNCoV2 and a SARS-CoV-2 vaccine provided by the campaign shall be at least 4 weeks.
9. Known hypersensitivity to any of the vaccine components (adjuvant or protein).
10. Administration of immunoglobulins and/or any blood products within the three months prior to the first dose of ABNCoV2 or planned administration during the study period.
11. Previous participation in a COVID-19 vaccine study.
12. Body Mass Index (BMI) >35 kg/m^2.
13. Pregnancy, lactation or intention to become pregnant during the study period.
14. History of drug or alcohol abuse interfering with normal functioning in the five years preceding enrolment.
15. Being an employee or student of the department of Medical Microbiology of the Radboudumc, or a person otherwise related to the investigator other than a professional relationship for clinical trial purpose only.
16. Any other condition or situation that would, in the opinion of the investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

4.4 Sample size calculation

COUGH-1 is an exploratory, Phase 1, first-in-human trial to evaluate the safety and tolerability of increasing doses of ABNCoV2 vaccine antigen with and without adjuvant, in healthy, COVID-19-naïve adults. Its outcome will inform dose and schedule for further clinical development. The overall objective is to assess safety and tolerability and identify an optimal immunogenicity-tolerability ratio. In case that the maximal dose of 70 µg is reached, a maximum number of 42 volunteers will be included.

The higher the probability of an adverse reaction (AR) in the vaccinated population, the smaller the sample of volunteers with at least one occurrence of the AR is. Table 3 shows probability of AR in vaccinees that can be detected with a given sample size at 90% power. A sample size of 6 can detect adverse reactions that occur in 32% of the participants with 90% power (with n=12, a prevalence of 17% can be detected). Assuming 42 volunteers are immunized, adverse reactions that occur in 4.7% of the volunteers (within the tested dose-range), can be detected with 90% probability.

Table 3. Probability of adverse reaction (AR)

Sample size (n)	Probability of AR (%)	Power
6	32	0.9
12	17	0.9
18	12	0.9
24	9.1	0.9
30	7.4	0.9
36	6.2	0.9
42	5.3	0.9
48	4.7	0.9
54	4.2	0.9
60	3.8	0.9

The names used to describe probability groupings of events (e.g. AR) customarily follow the convention: Very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000). Consequently, COUGH-1, as a Phase 1 first-in-human trial, is powered to detect very common and common events.

Immunogenicity

Dose escalation will be done in groups of 6 volunteers, starting with split groups, where half (n=3) of the volunteers receive the non-adjuvanted and half (n=3) the MF59-adjuvanted vaccine formulation. 14 days after administration of the first vaccination in Group 1-3, it will be decided whether the non-adjuvanted or the MF59-adjuvanted vaccine formulation will be used in the remainder based on superiority upon immunogenicity endpoints. The decision will be taken by the sponsor and the investigators and approved by a safety monitoring board based on all immunogenicity, safety and reactogenicity data that have been accrued until this time.

Assuming a sigmoid dose-immunogenicity relation, the optimal dose will be selected close to the upper asymptote, given it is still safe and well tolerated. To increase power at this threshold, the number of volunteers receiving the optimal dose ABNCoV2 will be doubled (total n=12 receiving 2 dosages).

A statistical analysis plan will be developed during preparation of the trial and updated based on pre-clinical findings as well as published data from other vaccine candidates. Immunological analyses will be developed using discovery and pre-clinical data generated during the project. Up to 7 reserve subjects will be recruited (1 reserve subject per dosage group).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Volunteers will sequentially receive an administration of non-adjuvanted or MF59-adjuvanted ABNCoV2 vaccination. All subjects will receive a second vaccination with the same dose and formulation 4 weeks following the first vaccination. In Group 1 (n=6), subjects will receive 6 µg ABNCoV2 intramuscularly, half of whom (n=3) will receive the non-adjuvanted vaccine formulation and the other half (n=3) will receive the MF59-adjuvanted vaccine formulation. In Group 2 (n=6), subjects will receive 12 µg ABNCoV2 intramuscularly, half of whom (n=3) will receive the non-adjuvanted vaccine formulation and the other half (n=3) will receive the MF59-adjuvanted vaccine formulation. In Group 3 (n=6), subjects will receive 25 µg ABNCoV2 intramuscularly, half of whom (n=3) will receive the non-adjuvanted vaccine formulation and the other half (n=3) will receive the MF59-adjuvanted vaccine formulation. In Group 4 (n=6), subjects will receive 50 µg non-adjuvanted or MF59-adjuvanted ABNCoV2 intramuscularly. In Group 5 (n=6), subjects will receive 70 µg non-adjuvanted or MF59-adjuvanted ABNCoV2 intramuscularly. The subjects in Group 6 (n=6) will receive the next lower dosage of the highest non-adjuvanted or MF59-adjuvanted ABNCoV2 dose achieved intramuscularly, and the subjects in Group 7 (n=6) will receive the highest non-adjuvanted or MF59-adjuvanted ABNCoV2 dose achieved intramuscularly. There will be no placebo group.

5.2 Use of co-intervention

Systematic use of a co-intervention is not planned (e.g. preventive use of non-steroidal anti-inflammatory drugs [NSAID]). In order to be eligible for the study, subjects must abide to the inclusion and exclusion criteria as described in section 4.2 and 4.3 of this protocol. Therefore:

- Subjects must pass a quiz (assessment of understanding) by scoring at least 80% correct on the multiple-choice quiz;
- Subjects may not chronically use immunosuppressive drugs or other immune modifying drugs within six months prior to study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expect to use such during the study period;
- Subjects must attend all study visits and must be reachable by phone throughout the entire study period from day -1 until 24 weeks following last vaccination (end of study);
- All subjects must use continuous adequate contraception for the duration of the study.

Female subject must undergo a pregnancy test at the inclusion visit. Females may not breastfeed during the study period;

- Subjects must refrain from blood donation to Sanquin or for other purposes throughout the study period;
- Subjects may not receive any investigational or non-registered product (drug or vaccine) other than the study product in the 30 days preceding enrolment or during the study period;
- Subjects may not receive any immunoglobulins and/or any blood products within three months preceding the first dose of ABNCoV2 or expect administration thereof during the study period;
- Subjects may not participate in any other clinical trial in the 30 days prior to start of the study or during the study period;
- Subjects must contact the trial physician if they experience any medical complaint throughout the entire study period;
- Subjects may not get immunization with any vaccines within the past four weeks or during the study period, with the exception of a licensed SARS-CoV-2 vaccine, given within the framework of the national SARS-CoV-2 vaccination campaign following ABNCoV2 administration. The time between last vaccination with ABNCoV2 and a SARS-CoV-2 vaccine provided by the campaign shall be at least 4 weeks.

5.3 Escape medication

No ABNCoV2-inactivating escape medication is available. The volunteers will not receive any premedication prior to the intramuscular injection of ABNCoV2 (e.g. nonsteroidal anti-inflammatory drugs – NSAIDs). In case of an anaphylactic reaction, the current version of the RUMC anaphylaxis Standard Operating Procedure (SOP) will be followed. The volunteers are allowed to take pain reliefers such as NSAIDs if required. They will be asked to document and report any use of concomitant medication during the trial.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

6.1.1 ABNCoV2 vaccine product

The ABNCoV2 vaccine is a sub-unit vaccine using a non-infectious virus-like particle (VLP) platform. A stabilized version of the receptor binding domain (RBD) of SARS-CoV-2 Spike

glycoprotein (S) is bound to a VLP, and through vaccination will be presented to the immune system in order to evoke a response resulting in protection from infection. Almost all vaccine candidates targeting SARS-CoV-2 and other members of the family *Coronaviridae* are based on S antigens and are optimized to achieve high invasion inhibition activities [14]. Proof-of-concept studies of S antigen-based vaccine candidates have shown high-level vaccine efficacy and transmission-blocking activity in dromedaries and other *Camelidae* [11-13], the main host of the Middle East respiratory syndrome coronavirus (MERS).

VLPs that display antigen on their surface are a technology to increase immunogenicity of soluble protein subunit vaccines [31]. Besides induction of better immune responses, memory and specificity of the response may also be improved. Since VLPs cannot replicate, they can be safely used in immunocompromised and senescent populations, two populations at risk for severe COVID-19. VLP-based vaccines have an extensive and excellent safety record. Examples of licensed and marketed VLP-based vaccines are hepatitis B (e.g. Engerix, HBVaxPro), and HPV vaccines (e.g. Gardasil, Gardasil 9, Cervarix).

In ABNCoV2 a novel VLP technology that allows covalent, directional, high density binding of different proteins on the VLP surface is used. The VLP consist of the Acinetobacter Phage 205 (AP205) capsid VLP (cVLP). AP205 does not infect eukaryotes (including humans). AP205 VLP was never used in humans so far but the closely related Phage Qβ phage VLP has been tested in clinical trials [25-27]. In model systems, AP205 VLPs increase and improve antibody responses when compared to soluble protein antigens [32-34].

The self-assembling AP205 VLP of the ABNCoV2 vaccine has modified capsid proteins that contain the split-protein Tag/Catcher system for antigen coupling [35]. Those binding sites distribute evenly across the outer surface of the particle and allow for fusion of large antigen proteins. The RBD of SARS-CoV-2 S is genetically engineered to bind to AP205 VLP through the split-protein Tag/Catcher system. Following coupling RBD antigens are presented on the VLP display platform at high density (72 spike RBD antigens per VLP). The RBD antigens are also unidirectionally oriented so that the ACE2 binding epitopes are positioned on the outermost surface of the complex, making it highly accessible for immune recognition.

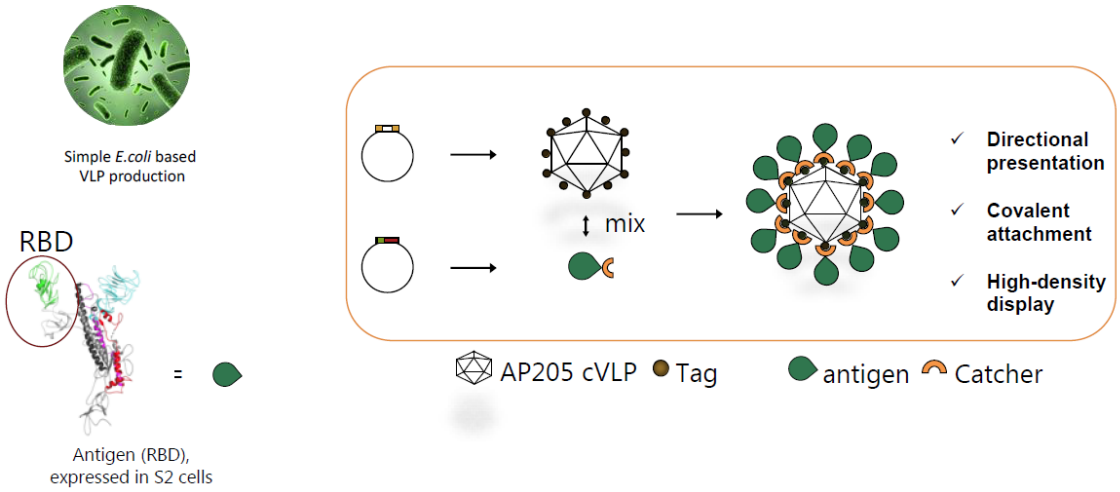

Figure 2. Structural Elements of ABNCoV2: Engineered AP205 VLP and Spike RBD Antigen. AP205: Acinetobacter Phage 205; cVLP: capsid virus-like particle; RBD: receptor binding domain; S2 cells: drosophila S2 cells; VLP: virus-like particle

6.1.2 MF59-adjuvant

Adjuvants are used to modulate type, strength and longevity of immune responses following vaccination. Adjuvants are particularly important for recombinant subunit vaccines that often do not lead to strong immune responses following intramuscular injection. VLP vaccines typically have better immunogenicity than soluble protein subunit vaccines. Therefore COUGH-1 will assess ABNCoV2 with and without MF59-adjuvant. MF59 is an oil-in-water emulsion containing squalene, polysorbate 80 and sorbitan trioleate. It is marketed in the Netherlands as part of the influenza vaccine Fluad/Fluad Tetra. It is approved for use in the elderly (>65 years) and has a favourable safety record. In influenza vaccines, MF59 can be used to reduce vaccine dose (dose-sparing), mainly acts through enhanced recruitment of immune cells to the injection site and has immune-stimulatory effects even in T helper cell deficient conditions [36].

6.2 Summary of findings from non-clinical studies

Preclinical data is described in detail in the investigator's brochure (IB) and Investigational Medicinal Product Dossier (IMPD). The immunogenicity of adjuvanted ABNCoV2 has been demonstrated in mice when formulated in the AddaVax, a bio-similar of GMP-produced MF59. BALB/c mice were vaccinated IM with soluble RBD, RBDn-CLP (ABNCoV2) or RBDc CLP (a VLP with the RBD bound to the c-terminus of the AP205 capsid protein instead of the n-terminal Tag/Catcher AP205). The ABNCoV2 vaccine and RBDc-CLP induced CoV-2 spike protein-specific IgG in all mice after a single vaccination and a booster vaccination increased the magnitude of the response. In contrast, soluble RBD required 2 administrations to induce

a detectable increase in vaccine-specific antibody concentration. In this study, doses (6.5 µg ABNCoV2) that corresponds to a human equivalent dose 10 to 20 times higher than 70 µg were used, which indicates a broad safety margin. Doses as low as 1 µg were also immunogenic.

Together with an increase in antibody concentration, a single vaccination with ABNCoV2 resulted in 100% neutralization of SARS-CoV-2 at a serum dilution of 1:80. The titre post booster immunization increased to 1:10,240. A correlation analysis between the ELISA antibody titres and neutralizing antibodies showed that there was a positive correlation between these measurements (Ks = 0.7152, p = 0.0461) in mice immunized with ABNCoV2 or RBDc-CLP, but not in the mice vaccinated with soluble RBD (Ks = 0.316, p = 0.4679). When compared with virus neutralization data generated in small animal models with other COVID-19 vaccine candidates, ABNCoV2 induced neutralizing antibody where highest (Figure 3).

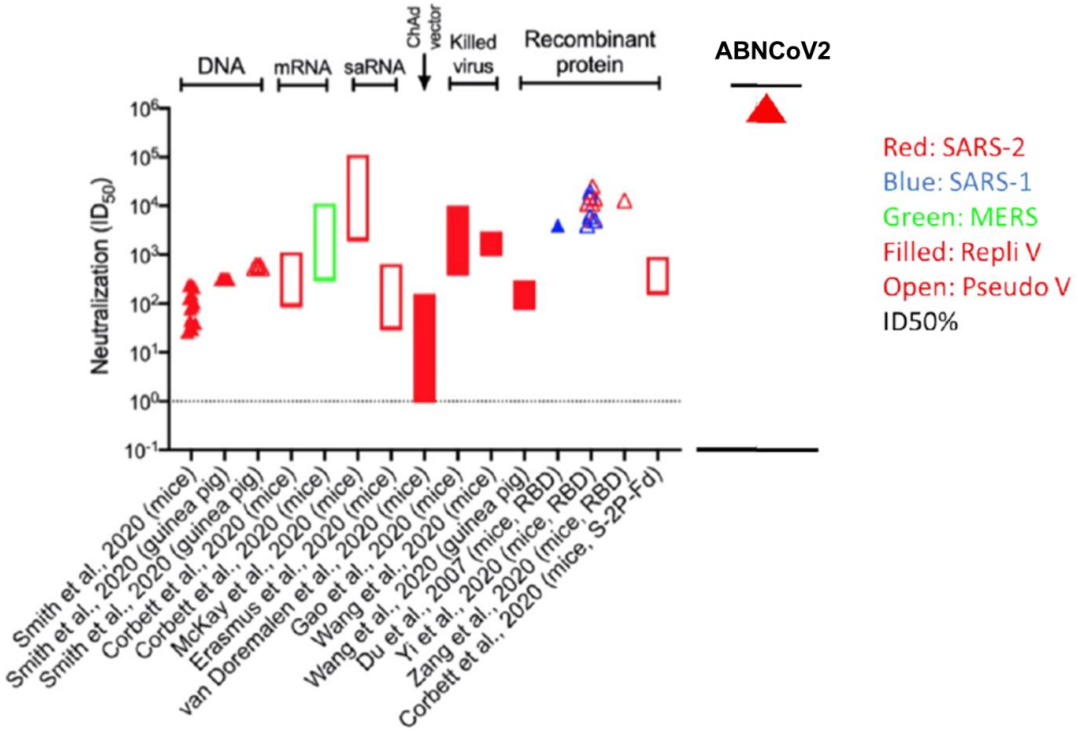

Figure 3. Neutralizing Antibody Levels Induced by Different COVID-19 Vaccines in Rodents.
DNA: deoxyribonucleic acid; ID50: infectious dose 50; mRNA: messenger RNA; Pseudo V: pseudovirus; RBD: receptor binding domain; Repli V: replicating virus; RNA: ribonucleic acid; saRNA: small activating RNA

A Good Laboratory Practice (GLP) compliant repeat dose toxicity study in New Zealand White (NZW) rabbits showed no abnormalities following two and three injections of 100 µg MF59-

adjuvanted ABNCoV2 that were considered a safety concern for progression in human trials. Compared to normal saline placebo, mild to moderate systemic and local reactogenicity was observed. All changes were reversible. In NZW rabbits, 100 µg translate to a human equivalent dose of >700 µg.

An ongoing nonhuman primate (NHP) challenge study in rhesus macaques has shown a robust increase in functional antibody titres following a single ABNCoV2 vaccination (15 and 100 µg ABNCoV2 with and without MF59) with a peak beyond 4 weeks. As in COUGH-1, a 1:1 formulation with MF59 was used. This study will go on to provide longer term immunogenicity and protection data, with a challenge ~5 months post vaccination. Immunogenicity and first safety results of the NHP study will be available before start of the trial, NHP challenge infections are scheduled later. Most likely, data will be available during the dose-escalation phase of COUGH-1.

6.3 Summary of findings from clinical studies

COUGH-1 is a first-in-human study. Preliminary early phase data as well as Phase 3 results of all published trials do not indicate a major risk related to the antigen [15-17, 20-22], e.g. vaccine-enhanced disease [37]. There are no past human experiences with the non-adjuvanted or MF59-adjuvanted ABNCoV2 vaccine. To date, two mRNA SARS-CoV-2 vaccines have been marketed in the Netherlands: Comirnaty and Moderna, see below in Table 4.

Table 4. Marketed SARS-CoV2 vaccines

Product number (EMA)	Name	Characteristics	Population	Very common adverse events (≥1/10)
EMEA/H/C/005735	Comirnaty (Pfizer/BioNTech)	mRNA vaccine	≥16 years old	Pain and swelling at the injection site, fatigue, headache, myalgia, arthralgia, chills, fever.
EMEA/H/C/005791	COVID-19 Vaccine Moderna	mRNA vaccine	≥18 years old	Lymphadenopathy, headache, nausea/vomiting, myalgia, arthralgia, pain and/or swelling at injection site, fatigue, chills, fever.

Although an AP205 cVLP vaccine like ABNCoV2 has not been previously tested in humans, there is extensive clinical experience with licensed VLP vaccines that protect against human papillomavirus (HPV) in addition to some early clinical experience with closely related Phage Qβ cVLP vaccines. cVLPs based on Phage Qβ (an RNA bacteriophage similar to AP205) have been examined in phase 1/2 clinical trials for indications of hypertension, asthma and smoking addiction, see Table 5. There is also a wealth of clinical data on influenza vaccines that are similarly adjuvanted with MF59. To date 270 million doses of recombinant VLP vaccines have been administered over the past 14 years. Overall VLP treatments have been shown to be generally safe and well tolerated, with reports of vaccine-related injection site and systemic reactions being most common.

Table 5. Clinical experience of Phage Qβ cVLP vaccines

NCT number	Indication	Intervention	Characteristics	Adverse events
NCT00710372 NCT00500786 NCT00701649	Hypertension	CYT006-AngQb Phage Qβ cVLP vaccine targeting angiotensin 2	Phase 1-2	Transient flu-like symptoms, local injection site reactions.
NCT00369616 NCT01280968	Nicotine (smoking cessation)	Phage Qβ cVLP vaccine	Phase 2	Transient flu-like symptoms, pyrexia, headache, nasopharyngitis, chills, myalgia and local injection site pain.

6.4 Summary of known and potential risks and benefits

The investigational study product, ABNCoV2, to be used in this study has not been administered previously to humans. All known risks and precautions described here are explained in detail in the informed consent documents.

6.4.1 Risk Assessment

The following section (Table 6) outlines the initial risk assessment and mitigation strategy for this study protocol.

Table 6. Risk Assessment

Important potential/identified risk	Data/rationale for risk	Mitigation strategy
Investigational study product (ABNCoV2 vaccine)		
Important potential risk: **First-in-human unknown risk**	There is no prior experience of ABNCoV2 vaccination in human subjects.	A dose escalating design will be used for Group 1 up till Group 5, starting with the lowest dose of 6 µg, which is lower than in all pre-clinical studies where no toxicity was observed (absolutely and when calculated as human equivalent dose – preclinical toxicity was assessed at a human equivalent dose of >700 µg). Subjects will be observed closely by qualified clinicians, and emergency care will be immediately available to subjects. A minimum of 6 days is required between product administration to the sentinel subjects of each group. In case that related serious related adverse events occur dose-escalation will be stopped.
Important potential risk: **Hypersensitivity (including anaphylaxis)**	As with other vaccines, hypersensitivity and anaphylaxis to one or several components of the vaccine can rarely occur.	Subjects who report allergic reaction against constituents of the vaccine in the past will not be recruited. All vaccinated volunteers will be observed closely for at least 60 minutes following administration of the product ABNCoV2 with appropriate medical treatment readily available in case of severe adverse events.
Local and systemic reactogenicity to intramuscular administration of ABNCoV2	ABNCoV2 vaccine has never previously been administered intramuscularly in humans. The risks associated with vaccination include local inflammatory reactions to the injected product, such as pain	Experienced and qualified medical personnel will administer the vaccination intramuscularly. Subjects will be informed about the local and systemic side effects that may occur and will be monitored closely for local and systemic adverse events. Subjects

	and swelling at the injection site. Systemic effects generally associated with vaccination may include flu-like symptoms, fever, chills, nausea, gastrointestinal symptoms, headache, malaise, myalgia and arthralgia.	will be advised to inform or call the study doctor immediately if they have any side effects.
Study procedures		
Pain when taking blood samples	Because of the necessity to obtain frequent blood sampling for laboratory safety analysis, determining the concentration of ABNCoV2-specific antibodies, and measuring cellular immune responses, there is a risk of feeling faint, or experience mild pain, bruising, irritation or redness at the site where blood was taken. In rare cases arteries or nervous tissue may be injured or a punctured vessel may occlude and induce inflammation of the surrounding tissue.	Experienced and qualified medical personnel will draw blood. The amount of blood to be taken for sampling will not be harmful to the subject's health. Total blood volume over the whole study period will be below <500 mL, less than the amount of a single whole blood donation.
Pregnancy risks		
Pregnancy and lactating females	Risks of the ABNCoV2 vaccine to unborn babies are unknown at this time; pregnant females will be excluded from this study.	Females are only eligible for participation if they agree to use efficient contraception during the study period and the pregnancy test during the inclusion visit is negative. In case effective contraceptives are not available the clinical team may provide them. Volunteer who becomes pregnant before the second immunization will not receive subsequent trial immunizations and will be withdrawn from any investigational product

		administration. Pregnant volunteers will not be withdrawn from the trial. Conditional on their agreement, they will be followed-up until the end of the pregnancy. Immunological and other exploratory blood samplings will be reduced to a minimum. Lactating females will be excluded from this study.
Risks to study personnel		
Needle-stick injuries	The principal risk in the clinical setting is the handling of needles that may be contaminated with blood or body fluids and the associated risk of acquiring a blood-borne pathogen (including hepatitis B and C viruses and human immunodeficiency virus (HIV)).	Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure. Subjects will be screened for hepatitis B and C and HIV prior to inclusion. Individuals positive for HIV, hepatitis B and C are excluded from the study.
Receiving another, authorized, SARS-CoV-2 vaccine		
Important potential risk: **Unknown risk(s) associated with administration of a different SARS-CoV-2 vaccine**	The risks associated with administration of a different SARS-CoV-2 vaccine after having received ABNCoV2, remain unknown.	Participants in the COUGH-1 trial offered an authorized SARS-CoV-2 vaccine as part of the official vaccination campaign during follow-up will be advised to receive it, although we will recommend participants not to do so earlier than 4 weeks after their second ABNCoV2 vaccination. This is to be able to attribute a majority of AE during follow up to ABNCoV2 administration and to decrease the risk of potential interaction between immune responses against ABNCoV2 and another SARS-CoV-2 vaccine. The risk of SARS-CoV-2 infection and, if infected, of severe COVID-19 is very low in the study population. Particularly, as it is expected that incidence will have dropped

		due to a combination of social distancing measures, mass vaccination and seasonal environmental factors. Volunteers who are offered an authorized SARS-CoV-2 vaccine during follow-up will be asked to attend additional study visits (before and after the vaccination), in order to document occurrences of (novel) adverse events, as well as changes in immunogenicity.

6.4.2 Benefit assessment

There is no direct benefit for study subjects from participation in the trial. It will be made clear that the vaccine is experimental and may not protect against COVID-19. Subjects may indirectly benefit from general medical evaluation and health screening procedures including testing for HIV, hepatitis B, and hepatitis C. Subjects will be informed about the results of the screening and if necessary, they will be referred to their primary physician where they will receive counselling and further medical attention earlier than if they did not know of their disease status. Society as a whole may benefit from the development of an effective COVID-19 vaccine, as this has the potential to improve the health of the worldwide population, as well as have extremely positive impacts on economic, social and health systems. Subjects will receive a financial compensation which is reasonable and in line with Dutch common practice (see 11.5).

6.5 Description and justification of route of administration and dosage

Enrolled participants will receive two intra-muscular injections of the ABNCoV2 vaccine in the deltoid muscle, preferably starting with the non-dominant arm at first vaccination, followed by the other arm at second vaccination. The administration site minimizes the risk that the injection goes into a blood vessel. In case of suspicion that a blood vessel is hit, the vaccine will not be administered. In case of trauma or anatomical abnormalities the same arm may be used for subsequent injections.

The vaccine will be administered on days 0 and 28. The trial is designed to evaluate the safety and tolerability in a staggered dose escalation of five dosages (6 µg, 12 µg, 25 µg, 50 µg and 70 µg). The initial starting dose proposed in this protocol is extrapolated from other VLP-type vaccines and pre-clinical data (see above). Four weeks after the first vaccination, a booster with the same dose and formulation as the first vaccination will be administered.

6.6 Dosages, dosage modifications and method of administration

Preparation of the syringe will be described in a separate pharmacy manual. All vaccinations will consist of the intramuscular injection with a syringe preferably starting with the non-dominant deltoid muscle. The syringe will be prepared by the pharmacy and delivered with the appropriate documentation. ABNCoV2 vaccine will be tested for the first time in humans. One objective of the study is to identify a dosage that optimizes the immunogenicity-tolerability ratio 14 days following first vaccination. MF59-adjuvanted and non-adjuvanted formulations will be tested in parallel in Group 1 up till Group 3 to detect superiority or futility of the MF59-adjuvanted against the non-adjuvanted formulation. If the adjuvanted vaccine formulation is superior based upon immunogenicity endpoints, whilst still remaining safe and tolerable (primary endpoints), subsequent Group 4 up till Group 7 will receive the adjuvanted vaccine. If not, Group 4 up till Group 7 will receive the non-adjuvanted vaccine. Dose-escalation will be performed until an optimal immunogenicity-tolerability ratio is reached with a ceiling of 70 µg. When immunogenicity dose-response is saturated (no further increase in immunogenicity and an acceptable tolerability profile) the highest reached and the next lower dosage will be repeatedly tested to increase power to detect adverse reactions at the optimal dose. Decision on adjuvant use and optimal dose will be made by the investigator and sponsor teams followed by a request of approval by a safety monitoring board (SMC), supported by analysis of tolerability, safety and immunogenicity data accrued up to the date of analysis.

6.7 Preparation and labelling of Investigational Medicinal Product

6.7.1 Presentation and formulation

ABNCoV2 (without adjuvant) will be supplied frozen in multidose glass vials closed with bromobutyl rubber stoppers, crimped with flip-off caps. The vial contents will be combined with MF59 adjuvant at the site pharmacy and distributed within the indicated shelf life of the formulated product.

The MF59 adjuvant will be supplied in glass-lined prefilled syringes that protect against light.

Prior to mixing ABNCoV2 with MF59 at the clinical trial site, the formulation of ABNCoV2 is supplied as 0.5 mg/mL +/- 0.1 mg/mL ABNCoV2, 1.2 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 136.9 mM NaCl, 2.7 mM KCl, 10.0 mM tris, 200 mM sucrose, pH 8.6. The drug product ABNCoV2 is a clear to opalescent liquid. Formulation will be done by the pharmacy at Radboudumc according to a study specific pharmacy manual. The procedure will be fully documented. The trial is not blinded. Hence, investigators and volunteers are aware of the content of the syringe used for injection.

6.7.2 Stability and storage

ABNCoV2 must be stored frozen at -20°C ± 5°C. MF59-adjuvant must be stored between 2°C and 8°C. It should not be frozen. ABNCoV2 drug product and MF59-adjuvant will be stored in a secure area with access limited to authorized study personnel, and will not be used for any other purpose other than the trial. ABNCoV2 formulated vaccines are stored at 2-8 °C and will be used within 24 hours. The storage conditions will be assessed during pre-trial monitoring visits. The storage temperature will be continuously monitored and recorded with calibrated temperature monitoring device(s). In case of temperature excursions, they will be reported in degrees Celsius. Any deviation from the recommended storage conditions will be immediately reported to the clinical trial monitor and the sponsor. The use of the impacted study vaccine must be interrupted until the sponsor has given authorization for its continued use.

6.7.3 Dose preparation and administration

Dose preparation, as described in the pharmacy manual, will be carried out by qualified personnel under controlled conditions. The ABNCoV2 vaccine product and adjuvant will be mixed prior to dosing at the clinical trial site pharmacy under the responsibility of a pharmacist according to a standard operating procedure (SOP). Syringes will be prepared under sterile conditions. Syringes contain either:

- 6 µg ABNCoV2
- 6 µg ABNCoV2 + MF59-adjuvant
- 12 µg ABNCoV2
- 12 µg ABNCoV2 + MF59-adjuvant
- 25 µg ABNCoV2
- 25 µg ABNCoV2 + MF59-adjuvant
- 50 µg ABNCoV2 +/- MF59-adjuvant
- 70 µg ABNCoV2 +/- MF59-adjuvant

From 50 to 6 µg two-fold serial dilutions will be used during the preparation of the vaccine product. Reported doses are rounded to the next lower integer for the two lowest doses.

All preparations will be diluted to give an injection volume of 0.5 mL. A pharmacy manual containing instructions for storage, preparation and handling of the vaccine will be provided to the pharmacist. Furthermore, trainings on the procedure will be performed before the first injection. Individual sterile single use 1 mL syringes for injection will be delivered to the person in charge of the injection. A separate sterile vanish point syringe will be used for each individual trial participant to prevent transmission of infectious agents. Before injection, the site of injection will be cleansed with an antiseptic and the entire content of each syringe will be used.

In case that a blood vessel is accidentally punctured, the vaccine is not injected and a novel syringe needs to be prepared. As for any injectable vaccine, appropriate equipment will be available in case of immediate allergic reactions. Enrolled participants will receive two intramuscular injections of the vaccine. The first will be given preferably in the non-dominant deltoid muscle, the second in contralateral muscle. The vaccine will be administered on day 0 and day 28. Caution will be taken to ensure that the injection does not go into a blood vessel (see above). In case of trauma or anatomical abnormalities a single arm or may be used. Side and site of injection will be documented.

6.7.4 Labelling

ABNCoV2 vaccine product and the MF59-adjuvant will be labelled and packaged according to applicable regulatory requirements. The labels will mention that the products are for investigational use only. The pharmacy at Radboudumc has a manufacturing authorization and may label the ABNCoV2 drug product and MF59 to fully comply with regulatory requirements. Details are given in the pharmacy manual.

6.8 Drug accountability

The drug product and adjuvants will be shipped to Radboudumc according to the relevant national and international regulations. The site pharmacist will maintain complete records of all study products received from the sponsor, an inventory, and an accountability record of the drug product and adjuvants for this study. The site pharmacist will also ensure the security of these documents. Vials will only be used for human administration within COUGH-1 and not for other *in vitro* or *in vivo* experimental studies. At the end of the study, the site will receive instruction from the manufacturer regarding the final disposition of any remaining study products. The person in charge of the product management will return a completed dispatch note to the sponsor, which will be attached to the package, as acknowledgement of receipt. The person in charge of product receipt will check that the cold chain was maintained during shipment. In case of a problem, the clinical trial monitor and the Sponsor must be alerted immediately. The acknowledgement of receipt will be dated and signed by the person in charge of product management. One copy will be kept archived; the other copy will be returned to the trial sponsor.

In accordance with all applicable regulatory requirements, the person in charge of the product management at the site must keep up to date the following inventory records:

- Receipt of product at the clinical trial site
- Treatment administered to each subject (including number of injection)
- Inventory of product at the clinical trial site
- Log of any unused or expired investigational product

These records should include dates, quantities, batch numbers, period of use or expiration dates (if applicable), and any unique code numbers assigned to the product subjects.
These records should document adequately that:

- The subjects were provided the doses specified by the protocol
- All products provided are fully reconciled

These records will be monitored and verified by the clinical trial monitor regularly, as part of the routine monitoring procedure.
Unused or open products will be either returned to the sponsor or designee at the end of the vaccination period together with the form "Return of unused or open products" or destroyed according to the sponsor's instructions.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study endpoints

8.1.1 Main study parameters/endpoints

- Primary safety endpoint: Number of at least possibly related Grade 3 adverse events (AE) and serious adverse events (SAE) from time of first administration of ABNCoV2 until the end of the follow-up period.
- Primary immunogenicity endpoint: Concentration of ABNCoV2-specific antibodies 14 days following first vaccination.

8.1.2 Secondary study endpoint

- Number and severity of at least possibly related solicited AEs within one week following administration of ABNCoV2.

8.1.3 Exploratory study parameters/endpoints

- Concentration of ABNCoV2-specific antibodies at baseline and during immunization and follow up.
- Inhibitory titre in invasion inhibition assay at baseline and during immunization and follow up.
- Cellular immune responses (T and B cell) at baseline and during immunization and follow up.
- Correlation of response vaccine to habitual sleep using the Pittsburgh Sleep Quality Index (PSQI) [38, 39].

8.2 Randomisation, blinding and treatment allocation

This will be an open-label trial to enable appropriate safety evaluation prior to proceeding to the next dose group. If the enrolment criteria are met, eligible subjects will be entered into the study. Randomization will not be used in this study. Subjects will be allocated based on logistical considerations (e.g. availability during follow up visits). The date/time of allocation and corresponding allocation group will be documented in the electronic case report form (eCRF).

8.3 Study procedures

It is the responsibility of the investigator to ensure only medically qualified study staff perform medical assessments and procedures which require medical training. Results of assessments should be reviewed with the subject, as appropriate, in a timely manner. It is also the responsibility of the investigator to ensure strict observance of the intervals between visits/procedures. These intervals are precisely defined for subjects in accordance with the protocol and are reflected in the study schedule outlined in section 8.3.19. In the context of the ongoing COVID-19 outbreak, the clinical investigator will follow current local and national guidelines concerning the mitigation of SARS-CoV-2 transmission and dedicated to minimizing the risk of exposure of study volunteers, study personnel, hospital staff & patients and the general public. This applies to all study visits.

8.3.1 Recruitment of subjects

The purpose of recruitment will be to obtain up to 42 eligible subjects for participation in this trial plus up to 7 reserve subjects (1 reserve subject per group). In order to recruit sufficient subjects, advertisements will be placed in prominent public places (e.g. as flyers), as well as on the University intranet. The study will be advertised on social media and local newspapers. The advertisement text will indicate a telephone number to call, an e-mail address, and a website to request further information. Interested subjects can register for the (digital) information meetings by filling in an online form which includes basic questions regarding their general state of health, use of medication, and participation in previous scientific studies. All subjects with an interest in participation will be advised to join one of the (digital) information meetings, where they will receive information on study participation and the rights and duties of subjects, as well as the corresponding documents (the study information sheet and study schedule, the application form, general information brochure on medical-scientific research from the Ministry of Health, Welfare and Sport, and the insurance text). If they are still interested after the information meeting and/or after reading the documents, an appointment for a screening visit will be made. The screening visit will be planned at least 24 hours after the subject receives the information sheet and informed

consent form. Since the information meeting is not mandatory, the documents can be e-mailed to subjects instead. Applicants will receive a pseudonymised screening number of two letters and a corresponding volunteer number as soon as they return their application form. This number will be used for source documents. This volunteer code will not contain any directly identifiable information.

8.3.2 Screening visit (visit 1)

The screening visit will take place within 28 days before the first vaccine administration. The screening appointment for individual subjects is planned at least 24 hours after the subject has received the information sheet and the informed consent form. The purpose of the screening visit is to provide and clarify study information to the volunteer and answer any questions the subjects may have, to obtain informed consent and to determine whether interested subjects are eligible for participation. The trial physician is responsible for providing the study information and performing the medical screening. A study nurse may draw venous blood, and collect vital parameters.

Upon arrival for the screening visit, the volunteer is given a medical questionnaire and a short quiz about the study to fill out. Subsequently, and prior to review of the questionnaire and quiz or any other screening activities, study staff will review the informed consent process with the volunteer. The possibility of withdrawal from the study, at any time and without any declaration of the reason, will be pointed out to the subjects. The investigator, or a person designated by the investigator, will fully inform the volunteer of all pertinent aspects of the study and individual consent will be documented by a signature. Subjects may only participate in screening if they have signed the informed consent form. All subjects must consent to a SARS-CoV-2, HIV, hepatitis B and hepatitis C serological screening, urine toxicology and for females also a pregnancy test. Subjects who sign the informed consent will undergo complete screening. After subject consents, the below activities will occur:

- The study quiz will be reviewed and if any incorrect answers were given, these will be given additional attention. If the score on the quiz was <80%, the subject will be given the opportunity to retake it.
- Patient history: the answers to the medical questionnaire are discussed with the volunteer and clarified where needed. The subject will be further interviewed to collect demographic data, medical history including details of any chronic or recurrent medical and psychiatric conditions and use of adequate contraception;
- Study information: the study schedule and study rules are discussed with the subject and any questions are answered;
- A physical examination including vital signs, height and weight will be performed;

- Blood specimens will be collected for routine clinical laboratory testing of biochemical and hematological parameters, SARS-CoV-2 antibodies, as well as HIV, hepatitis B and hepatitis C serological screening;
- A urine specimen will be collected for toxicology screening and for females a pregnancy test if the subject is of childbearing potential;
- If necessary, consent to inform a medical specialist of study participation, will be signed by the subject and sent after screening.

If physical examination, vital signs or laboratory values are out of the normal range, a repeat measure may be obtained as deemed necessary by the investigators. The medical history, physical examination, and laboratory findings for subjects will be recorded in the source screening data documents. All subjects will be asked to supply a phone number of a partner or roommate who may be contacted in case of emergency. Concomitant medications is recorded at all study visits. All results of the screening will be reviewed with the subject. Subjects are informed by phone if they have satisfied all the inclusion criteria. If clinically significant abnormalities are identified during screening, subjects will be referred to their primary health provider or appropriate medical center. If identified during the study, subjects may be asked to return to the study site for further evaluation, including clinical evaluation and repeat laboratory testing as warranted. If more than 28 days have passed since screening before the intended day of first vaccine administration, the subject will be rescreened.

8.3.3 Inclusion visit (visit 2)

Subjects meeting the eligibility criteria during screening (section 4.2 and 4.3) will be invited back for enrolment into the study at the inclusion visit, which will occur prior to the planned administration day. Baseline assessments will be taken on the inclusion day. For each subject, study start (day 0) will be defined as the day of first ABNCoV2 administration. For subjects that do not show up for inclusion visit (visit 2) or first inoculation visit (visit 3), alternate subjects may be included. At study inclusion, the following activities will occur:

- Patient history will be taken and all AE that have occurred since screening will be noted. Only subjects who still meet the inclusion criteria will be included to receive ABNCoV2;
- A nasopharyngeal swab for SARS-CoV-2 PCR;
- Blood specimens will be collected for routine clinical laboratory testing of biochemical and hematological parameters;
- Non-invasive sampling of saliva and mucosal secretions of the respiratory organs will be done to compare presence of antibodies at the site of infection to serum.

- A urine specimen will be collected for toxicology screening and for females a pregnancy test if the subject is of childbearing potential;
- Subject will be issued a thermometer and symptom diaries to record any local and systemic symptoms and medication use;
- Subject will be issued an emergency notification card that details their participation in the study and provides contact phone numbers of the investigators.

8.3.4 ABNCoV2 inoculation visits (visits 3 + 9)

The purpose of these visits is to inoculate enrolled subjects with ABNCoV2. On the day of the first ABNCoV2 administration, the subjects will be admitted for approximately 4 hours. On the day of the second ABNCoV2 administration, the subjects will be admitted for approximately 1 hour. Subjects will be briefly assessed regarding any new medical events since the inclusion visit or most recent follow-up visit. Temperature, blood pressure, and pulse rate will be recorded. To confirm eligibility, results of all assessments will be reviewed. If the subject is still eligible for participation, the following activities will occur as detailed below:

- ABNCoV2 product will be prepared as described in the pharmacy manual by pharmacy staff based on group assignment.
- ABNCoV2 will be administered by intramuscular injection in the deltoid muscle (if possible into alternating sides, starting with the non-dominant arm). Subjects will be observed directly for at least 60 minutes after administration.
- Subjects may leave the unit at the Radboud Technology Center for Clincial Studies (RTCCS, hereafter 'study center') after 4 hours on the day of the first ABNCoV2 administration and after 1 hour on the day of the second ABNCoV2 administration. Subjects return the next day to the clinical research center for the 24-hour blood sampling time point.
- Vital signs, AE (solicited and unsolicited) and blood will be collected at each time point as indicated in section 8.3.19.
- A list of solicited local and systemic symptoms will be reviewed with the subject prior to and after administration. If the occurrence of an AE or use of medication is confirmed by the study physician, it is recorded in the clinical trial database.
- The PSQI questionnaire about sleep behaviour will be filled in by the subject.
- After leaving the study center, subjects will be asked to examine the site of injection and record local signs and symptoms for seven days, including bruising, erythema, swelling or induration. Subjects will be asked to measure their oral temperature daily until day 7 after each administration. Subjects will be asked to record any AEs in the memory aid booklet until day 196 of the

study. At the end of the study, the memory aid will be collected and kept as source data with the subject's study file.

All inoculations are performed by a trained nurse under the supervision of one of the clinical investigators. Another clinical investigator will be on call in case of emergency. Local hospital procedures will be followed. Study subjects will be monitored at the RTCCS Unit on the days of ABNCoV2 administration. Follow-up visits will take place as described below.

8.3.5 Follow-up visits after ABNCoV2 administration (visit 4-8 and 10-16)

Follow-up visits will be carried out on an outpatient basis at the study center. Subject will be asked to examine the site of injection and record local signs and symptoms in their memory aid booklet (including bruising, erythema, swelling or induration) for seven days after injection as well as systemic symptoms. Subjects will also be asked to measure their body temperature in the mornings for 6 consecutive days after ABNCoV2 administration. The subject diary will be reviewed at each study visit and used as a basis for discussion of possible local and systemic AE or medication use. If the occurrence of an AE or use of medication is confirmed by the study physician, it is recorded in the clinical trial database. At the end of the study, the diary will be collected and kept as source data with the subject's study file. Follow-up visits *not* requiring blood collection may be carried out by telephone instead of at the study center. During the study period subjects will be instructed to call the trial physicians at any time if they experience significant symptoms. If clinically significant abnormalities are identified during the study, subjects may be asked to return to the study center for further evaluation, including clinical evaluation and repeat laboratory testing as warranted. The trial physician can decide to initiate any additional diagnostics (including safety laboratory evaluations) at all times. For unexpected laboratory abnormalities, the laboratory test may be repeated. If there is any ambiguity regarding these results, a trial physician will discuss the case with the local safety monitor.

8.3.6 Unscheduled visits

Subjects may need to present to the study center for an unscheduled visit should they experience any AE that requires evaluation by the trial clinician. Data for any examinations performed on the subject at an unscheduled visit must be recorded in the eCRF. If an unscheduled visit is performed, the procedures for the next following visit should not be made earlier than scheduled above.

8.3.7 Visits regarding vaccination with licensed SARS-CoV-2 vaccine

Subjects who are offered an authorized SARS-CoV-2 vaccine during the follow-up period will be allowed to receive it. Preferentially, late following their second ABNCoV2 vaccination (not earlier than 4 weeks). This is to be able to attribute a majority of AE during follow-up to ABNCoV2 administration and to decrease the risk of potential interaction between immune responses against ABNCoV2 and another SARS-CoV-2 vaccine. The risk of SARS-CoV-2 infection and, if infected, of severe COVID-19 is very low in the study population. Particularly, as it is expected that incidence will have dropped due to a combination of social distancing measures, mass vaccination and seasonal environmental factors. Subjects who are offered an authorized SARS-CoV-2 vaccine during follow-up will be asked to attend additional voluntary study visits before and after the vaccination. Subjects will be compensated 25 euros per extra on-site follow-up visit.

8.3.8 Medical history

The trial clinician will review the medical history of potential study subjects during the screening visit. The starting point will be a medical questionnaire the subjects have filled out. Particular attention will be paid to:

- Current or recent (within the previous two weeks) acute respiratory illness with or without fever, including symptoms associated with COVID-19;
- COVID-19 infection in the past;
- Recent receipt of immune globulin or other blood products, or injected corticosteroids or other immune modulator therapy within the previous six months;
- Hypersensitivity of any kind;
- Clinically relevant history of cardiovascular, renal, gastrointestinal, hematological, dermatological, endocrine, neurological or immunological diseases;
- Known or suspected immunologic function impairment of any kind and/or known HIV infection, hepatitis B or hepatitis C infection;
- Mental illness;
- Tobacco, alcohol, or drug use;
- Medication use in the past 6 months;
- For women, pregnancy and contraceptive use and/or history of surgical sterilisation. For males, use of contraception;

8.3.9 Physical examination

A physical examination will be performed during screening, including the examination of general appearance, skin, neck, eyes, throat, lungs, heart, abdomen, back and extremities, and a routine vascular and neurological examination. During follow-up visits a focused physical examination will be performed if deemed necessary by the trial physician.

8.3.10 Height and weight

Height (cm) and body weight (to the nearest kilogram [kg] in indoor clothing, but without shoes) will be measured at the screening visit. Body mass index (BMI) will be calculated using the following formula: BMI = Body weight (kg) / [height (m)]^2 and converted to an integer.

8.3.11 Vital signs

Vital signs including body temperature (degrees Celsius), blood pressure (BP, millimeters of mercury) and pulse measurements (beats per minute) will be recorded at the screening visit. Additional measurements will take place at the discretion of the physician. Systolic and diastolic BP will be measured while the subject is sitting, with the back supported and both feet placed on the floor, using an validated automated device, with an appropriately sized cuff. In case the cuff sizes available are not large enough for the subject's arm circumference, a sphygmomanometer with an appropriately sized cuff may be used. If vital signs are out-of-range at screening or baseline, the investigator may obtain two additional readings, so that a total of up to three consecutive assessments are made, with the subject seated quietly for approximately five minutes preceding each repeat assessment. At least the last reading must be within the normal range in order for the subject to qualify. Additionally, subjects will be given a thermometer to measure their oral temperature on a daily basis for one week after ABNCoV2 administration, which they will collect in their study diaries.

8.3.12 Laboratory evaluations

During the study, blood samples will be drawn for screening, safety and research purposes. The blood sampling schedule is shown in the flowchart (section 8.3.19). Following universal precautions, blood will be collected by venipuncture into vacutainer tubes. Blood specimens will be affixed with coded labels that link the specimen to the subject, specimen type, specimen collection date, and time-point. The specimen collection tube must be appropriate for the type of specimen required. The cumulative blood draw for each subject over the entire course of study participation will be ≤500 mL (the approximate amount of one blood donation). Biological safety parameters will be measured in plasma or serum samples at the central laboratory of the Radboudumc. In the case a laboratory assessment is outside the reference range, a decision regarding whether the result is of clinical significance or not shall be made by the

investigator and shall be based, in part, upon the nature and degree of the observed abnormality. The assessment may first be repeated for confirmation. All abnormalities will be documented in the source documents, including clinical considerations.

Complete Blood Count (CBC)

Complete hematology tests, including hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (including neutrophils, basophiles, eosinophils, monocytes and lymphocytes) and platelet count will be measured when appropriate. Complete hematology will be done at screening, at 24 hours and days 7, 27, 29, 35 and 196. HbA1C will only be measured at screening.

Clinical Chemistry

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, sodium, potassium, bilirubin, yGT and LDH will be measured at screening, at 24 hours and days 27, 29 and 196. Glucose, cholesterol and triglyceride will only be measured at screening.

In addition to blood samples, non-invasive sampling of saliva and mucosal secretions of the respiratory organs will be done to compare presence of antibodies at the site of infection to serum.

8.3.13 Urine toxicology analysis

A midstream urine sample (approx. 30ml) will be obtained, in order to avoid contamination with epithelial cells and sediments, to allow proper assessment. A toxicology screening will be performed at inclusion (visit 2). A positive test for amphetamines and cocaine is a reason for exclusion.

8.3.14 Pregnancy test

A midstream urine sample (approx. 30ml) will be obtained and assessed by commercially available hCG urine tests. This test will be done at screening (visit 1), inclusion (visit 2) and the day before the second vaccination (visit 8).

8.3.15 Discontinuation of study procedures

Participants have the right to decline study procedures for any reason and at any time during the study. If a subject declines study procedures, this will be recorded as a study deviation and the reason will be clearly documented in the source document. The subject will be encouraged to complete all remaining safety related follow-up visits and procedures through the entire study period, including unscheduled visits. The reason for all discontinuations will be documented in source documents and the eCRF.

8.3.16 Assay to determine concentration SARS-CoV-2-specific antibodies

Antigen-specific total IgG titers will measured by enzyme-linked immune-sorbent assay (ELISA). Briefly, 96-well plates (Nunc MaxiSorp) will be coated overnight at 4°C with 0.1µg/well recombinant ExpreS2 produced SARS-CoV-2 Spike (35-1227) protein in PBS. After blocking for 1 hour at room temperature (RT) using 0.5% skimmed milk in PBS human serum prediluted 1:100 in blocking buffer will be added to the plate in a 3-fold dilution, followed by incubation for 1 hour at RT. Plates will be washed three times in PBS in between steps. In order to measure total serum IgG or Isotype specific IgGs, Horseradish peroxidase (HRP) conjugated goat anti-human IgG, IgG1, IgG2, IgG3 or IgG4 diluted 1:1000 in blocking buffer will be added followed by 1 hour incubation at RT. Plates were developed with TMB X-tra substrate (Kem-En-Tec, 4800A) and absorbance was measured at 450nM. Data were collected on Microsoft excel (Microsoft 360, excel 2016) and analysed using graphpad prism (San Diego, USA, version 8.4.3). Data will be represented by Area under the curve, IC50 and Endpoint titres.

8.3.17 Virus neutralization assay

Invasion inhibition assays are based upon the method described previously [40]. Briefly, SARS-CoV2, Freiburg isolate, FR-4286 and Leiden-001, will be propagated in VeroE6 expressing cells expressing human TMPRSS2 (VeroE6-hTMPRSS2) with a multiplicity of infection (MOI) of 0.05. Supernatant containing new virus progeny will be harvested 72h post infection, and concentrated on 100kDa Amicon ultrafiltration columns (Merck) by centrifugation for 30 minutes at 4000 g. Virus titer will be determined by $TCID_{50\%}$ assay and calculated by Reed-Muench method [41]. Participant serum will be heat-inactivated (30 min, 56 °C), and prepared in a 2-fold serial dilutions in DMEM (Gibco) + 2% FCS (Sigma-Aldrich) + 1% Pen/Strep (Gibco) + L-Glutamine (Sigma-Aldrich). Sera will be mixed with SARS-CoV-2 at a final titer of 100 $TCID_{50}$/well, and incubated at 4°C overnight. No serum and a no virus (uninfected) controls will also be established. The following day virus:serum mixtures will be added to 2 x 10^4 Vero E6 TMPRSS2 cells seeded in flat-bottom 96-well plates, and incubated for 72h in a humidified CO_2 incubator at 37 ˚C, 5% CO_2, before fixing with 5% formalin (Sigma-Aldrich) and staining with crystal violet solution (Sigma-Aldrich). The plates will read using a light microscope (Leica DMi1) with camera (Leica MC170 HD) at 4x magnification, and cytopathic effect (CPE) scored.

8.3.18 Safety assessments

Planned safety assessments will provide the data for active monitoring of investigational product safety during conduct of the trial and for the primary reactogenicity and safety endpoints. Subjects will be observed directly for 60 minutes following product administration.

AEs will be recorded prior to administration and at all follow-up visits. Clinical laboratory results assessed as AEs will be scored for severity using US FDA Toxicity Tables [30].

8.3.19 Time and event procedures

Type of contact	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15	Visit 16
Study day		**I1-3**	**I1**	**I1+1**	**I1+2**	**I1+7**	**I1+14**	**I2-3**	**I2**	**I2+1**	**I2+2**	**I2+7**	**I2+14**	**I2+42**	**I2+91**	**I2+168**
Time after first administration	**D-28 to D-1**	**D-3**	**D0**	**D1**	**D2**	**D7**	**D14**	**D25**	**D28**	**D29**	**D30**	**D35**	**D42**	**D70**	**D119**	**D196**
Informed consent	●															
Inclusion/exclusion criteria	●	●						●								
Demographic data and medical history	●															
Sleep behaviour questionnaire			●													
Physical examination[1]	●	●[1]	●[1]	●[1]		●[1]	●[1]	●[1]	●[1]	●[1]		●[1]	●[1]	●[1]	●[1]	●[1]
Blood pressure, temperature, pulse[2]	●	●[2]	●[2]	●[2]		●[2]	●[2]	●[2]	●[2]	●[2]		●[2]	●[2]	●[2]	●[2]	●[2]
Record any concomitant medications	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
HIV, HBV, HCV	●															
Drugs screening (urine)		●														
Pregnancy test (urine)	●	●						●								
Distribution of emergency notification card, memory card and thermometer		●														
SARS-CoV-2 PCR (swab)		●						●								
SARS-CoV-2 antibodies	●						●	●					●	●	●	●
ABNCoV2 inoculation[3]			●[3]						●[3]							

Solicited AEs (days 0–7 and 28-35)			•	•	•	•			•	•	•	•				
Unsolicited AEs (days 0-196)			•	•	•	•	•	•	•	•	•	•	•	•	•	•
Complete blood count[4]	•			•		•		•		•		•				•
Biochemistry tests[5]	•			•				•		•						•
PBMC[6]		•				•		•				•		•		•
Serum banking[6]		•						•								•
Safety Report[7]							•									•
End of study visit																•
Cumulative blood volume (mL)[8]	≤500 mL															

1. A physical examination including height and weight and vital parameters will be performed at the screening visit. Additional measurements will take place at the discretion of the physician.
2. Vital signs including body temperature, blood pressure and pulse will be recorded at the screening visit. Additional measurements will take place at the discretion of the physician.
3. Subjects will receive the first dose of ABNCoV2 on day 0, followed by a booster with the same dose and formulation on day 28.
4. CBC test includes: hemoglobin, hematocrit, platelets, red blood cell count, MCV, MCH, white blood cell count + differentiation. Additional at screening: HbA1C.
5. Biochemistry test includes: creatinine, urea, sodium, potassium, bilirubin, yGT, AST, ALT and LDH. Additional at screening: cholesterol, triglyceride, and glucose.
6. Blood will be collected for PBMC's and serum banking. Saliva will be collected in parallel.
7. Safety report: A safety report for Groups 1-3 is prepared by the clinical investigator once the final subject in Group 3 has completed 14 days of follow-up. A safety report of all the safety data is made after the close-out visit.
8. Sampled blood volume will be tracked over the whole study period to ensure that ≤500 mL (equivalent to one whole blood donation) will be sampled in total and all safety analyses can be done throughout.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. Subjects can also be withdrawn from the study procedures at the discretion of the clinical investigator or the local safety monitor for urgent medical reasons or if exclusion criteria are met. The following reasons may lead to withdrawal of individual subjects:

- Withdrawal of informed consent by volunteer;
- Any SAE;
- Discovery of a condition or medication that according to clinical judgment of the investigator is considered as a definite contraindication to proceeding with the study procedures;
- Immunosuppressant or other immune-modifying drugs administered chronically (i.e., more than 14 days in total) during the study period. Inhaled and topical corticosteroids and oral anti-histamines exempted;
- Immunoglobulin and/or any blood products administered during the study period;
- Pregnancy;
- Complete lost to follow-up;
- Ineligibility (arising during the study or retrospectively, having been overlooked at screening);
- If the investigator or safety monitor believes that continuation would be detrimental to the subject's well-being;
- Volunteer non-compliance with study requirements;
- Any other protocol deviation that results in a significant risk to the subject's safety.

8.4.1 Handling of withdrawals

For subjects lost to follow-up (i.e. those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), extensive effort (i.e. documented phone calls and e-mails) will be undertaken to locate or recall the volunteer or at least to determine his or her health status. The investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject. In case of premature withdrawal for any reason, the investigator will exert his/her best effort to:

- Update any ongoing AE/SAEs that remained ongoing at the time of the subject's last visit prior to withdrawal.
- Conduct an interview to determine if the subject has had any reaction or AE (serious or non- serious) since the last visit. Where possible, the investigator should visibly or physically assess any reported adverse reaction or AE and document whether it led to the withdrawal.

- Conduct a physical examination.
- Collect blood for biochemical and hematological clinical laboratory parameters.
- Document the reason for premature withdrawal on the CRF.
- Update the subject's contact information.

8.4.2 Withdrawals at specific time-points

During screening

If the investigator ascertains that the subject does not meet the inclusion/exclusion criteria after the subject signs the informed consent form, the investigator only needs to note the reason for subject exclusion on the screening log.

As a result of erroneous inclusion

If a subject who does not meet the inclusion/exclusion criteria is inadvertently included in the trial, the investigator will terminate the subject's participation in the trial, the sponsor will be informed immediately prior to administration of study product and a protocol deviation will be completed. In the event that the study product has already been given, the subject will be asked to complete all remaining safety related follow-up visits and procedures through the last study visit. However, the subject's data will be excluded from the per-protocol analysis.

As a result of withdrawal of informed consent

If a subject withdrawal occurs for any reason, the investigator must make every effort to determine the primary reason for a subject's withdrawal from the study and record this information in the study file. However, in accordance with the principles of the current version of the Declaration of Helsinki, it is acknowledged that a subject does have the right to withdraw from the study at any time and for any reason and is not obliged to give her or his reasons for doing so. With permission of the subject, all data generated before withdrawal will be included in final study analysis, unless it is compromised or the subject objects. Blood samples collected before withdrawal will be used/stored unless the subject specifically requests otherwise.

As a result of occurrence of an adverse event/serious adverse event

If any subject develops an AE/SAE leading to premature withdrawal, the event will be fully followed-up. For premature withdrawal in connection with the emergence of an AE/SAE that is considered to be clinically relatively favourable (for example, the diagnosis is known, and it is expected to be resolved completely within a week), intermittent follow-up may be accepted as long as the plan for follow-up of the event is fully described in the notes section of the subject's CRF. Follow-up visits will be carried out for all subjects who are withdrawn due to occurrence of an AE or in connection with a change in any other safety indicator (vital sign or clinical laboratory result). All data generated before withdrawal will be included in the

final study analysis, unless it is compromised or the subject objects. Blood samples collected before withdrawal will be used/stored unless the subject specifically requests otherwise.

As a result of loss to follow-up

To prevent loss to follow-up, subjects will be reminded by phone, email, or text message of their next study visit. In the event of a missed visit, subject will be contacted by phone within 1 day. A subject who misses two consecutive visits and cannot be reached or located after 5 attempts will be considered lost to follow-up. Efforts to contact the subject will be documented in source documents. Any subject who fails to attend the final study visit will also be classified as lost to follow-up. There will be no replacement for subjects who are lost to follow-up. All data generated before loss to follow-up will be included in final study analysis with subject's permission, unless it is felt that the data is compromised. Blood samples collected before loss to follow-up will be used/stored unless the subject specifically requests otherwise.

8.5 Replacement of individual subjects after withdrawal

If an assigned subject does not present on the day of ABNCoV2 study product administration or elects to withdraw the consent on the day of study product administration, one of the reserve subjects will replace the withdrawn subject. Subjects who have received the first dose of ABNCoV2 cannot be replaced.

8.6 Follow-up of subjects withdrawn from treatment

A subject may end his or her participation in the study and still be followed up for safety, unless the subject chooses to have complete withdrawal of the consent for further participation in any trial procedures. If a subject chooses to withdraw from the study, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal and complete the study termination eCRF.

8.7 Premature termination of the study

The study may be discontinued by the sponsor:

- On advice of the local safety monitor
- On advice of the Safety Monitoring Committee (SMC)
- On advice of the clinical investigator
- On advice of the METC (CCMO)

The investigators, local safety monitor, SMC, METC or sponsor may decide to put the study on hold based on AEs, pending discussion with the sponsor, SMC, METC, local safety monitor or investigators. Following discussion, it may be decided to terminate the study.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

AE are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or trial procedure. All AE reported spontaneously by the subject or asked about or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A SAE is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have, based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a SAE.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the SAE.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    a. Summary of Product Characteristics (SPC) for an authorised medicinal product;
    b. Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or *ToetsingOnline* is sufficient as notification to the competent authority. The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.4.1 Adverse event data collection

Signs and symptoms will be recorded in study diaries and reviewed during admission at the clinical trial research center, at all follow-up visits, and whenever a trial volunteer reports signs or symptoms to the trial physician between visits.

Solicited adverse events

After the administration of ABNCoV2, the following local and systemic AE will be solicited until 7 days after administration:

- *Local AE: pain, tenderness, erythema and induration at injection site*.
- *Systemic AE: headache, fatigue, fever, drowsiness, chills.*

The time points that these symptoms are solicited is indicated in section 8.3.19.

Unsolicited adverse events

Unsolicited AE occurring through 196 days following the first ABNCoV2 administration will be collected during the entire study period.

9.4.2 Recording of adverse event data collection

The trial physician will train subjects on the use of memory aid booklets for self-assessment of solicited local and systemic AE through 7 days post- administration of ABNCoV2, and for self-assessment of unsolicited AE and concomitant medications during the entire study period. The trial physician will review and discuss with the subject all recorded events to ensure appropriate documentation and scoring prior to transferring of the diary card record to the eCRF.

If known, the trial clinician will record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values. If the signs and symptoms are considered

unrelated to an encountered syndrome or disease they should be recorded as individual AEs. If a primary AE is recorded, events occurring secondary to the primary event should be described in the narrative description of the case (e.g. primary AE = Orthostatic hypotension; secondary event may be fainting, head trauma, etc.). In case of hospitalizations for surgical or diagnostic procedures, the pre-existing condition should be recorded as the SAE, not the procedure itself.

All adverse events/reactions (solicited and unsolicited), noted by the investigators will be accurately documented in the case report form by the investigators. For each event/reaction the following details will be recorded:

1. Description of the event(s)/reactions(s)
2. Date and time of occurrence
3. Duration
4. Intensity
5. Relationship with the intervention
6. Action taken, including treatment
7. Outcome

In addition, symptoms will be ranked as (1) mild, (2) moderate, or (3) severe, depending on their intensity according to the following scale:

Mild (grade 1):	awareness of symptoms that are easily tolerated and do not interfere with usual daily activity.
Moderate (grade 2):	discomfort that interferes with or limits usual daily activity.
Severe (grade 3):	disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest.

The investigator will attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

If an AE changes in intensity during the specified reporting period, a new description of the AE will be added. Interrupted AEs are registered as one AE if the interruption is <24 hours. When an AE/SAE occurs, it is the responsibility of the investigators to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event. The investigators will then record all relevant information regarding an AE/SAE on the CRF or SAE Report Form.

9.4.3 Assessment of causality

The investigators are obliged to assess the relationship between study procedures and the occurrence of each AE/SAE. The investigators will use clinical judgment to determine the relationship. Alternative causes, such as natural history or the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event will be considered and investigated. The relationship of the AE with the study procedures will be categorized as:

Definitely:	administration of the IMP is the cause, another etiology causing the AE is not known.
Probable:	administration of the IMP is the most likely cause: however, there are alternative reasonable explanations, even though less likely.
Possible:	there is a potential association between the event and administration of the IMP, however, there is an alternative etiology that is more likely.
Unlikely:	a relationship to the administration of IMP is unlikely, however, it cannot be ruled out.
Not related:	a relationship to the administration of the IMP cannot be reasonably established; another etiology is known to have caused the AE or is highly likely to have caused it.

When a regulatory authority requests a binary classification (related vs. unrelated), definitely, probably and possibly related are considered to be “related”, while not related and unlikely related are considered to be “unrelated”. Thus, an intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The investigator will use clinical judgment to determine the relationship.

9.5 Safety Monitoring Committee

An independent SMC composed of three independent individuals will be appointed. The SMC will include a local safety monitor, and two experts nominated by the PI. The SMC will be established for the purpose of monitoring the study, to discuss and approve the investigator and sponsor’s decision on further dose escalation and adjuvant use in groups 4 and above, as well as to provide independent, non-binding advice on safety and ethics. The responsibilities and procedures of the SMC members are defined in the SMC Charter.

The advice(s) of the SMC will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the SMC, the sponsor will send the advice to the

reviewing METC, including a note to substantiate why (part of) the advice of the SMC will not be followed.

9.5.1 Local safety monitor

For this study, a local safety monitor will be appointed, who will be involved in the review of severe and serious AE and volunteer safety. She/he is independent of the sponsor and the investigator. The local safety monitor is notified of all SAEs, as well as at least all grade 3 AE probably or definitely related to ABNCoV2 administration and persisting at grade 3 for >48 hours.

9.5.2 Safety Meetings

The SMC will review safety data upon completion of ≥14 days follow-up after the first immunisation in all subjects up to and including group 3. The chair of the SMC will determine whether a (digital) meeting will be held, or whether a recommendation from all members may be formalized through e-mail. In addition, safety data for all participants will be assessed by the SMC at the end of the study. An ad hoc SMC meeting may be convened at any time or at the request of the PI or local safety monitor to review safety data from subjects and/or groups who meet any of the holding rules as specified in the protocol and SMC charter, or if otherwise deemed necessary.

9.5.3 Safety Reports

A safety report will be prepared by the clinical investigator for review by the committee prior to each scheduled data review. These reports will provide at a minimum the following information:

- Accrual data and subject status data with regard to completion of/discontinuation from the study.
- Summaries of solicited AEs, classified by severity.
- Unsolicited AEs (including SAEs), categorized by MedDRA coding, severity and relatedness to study vaccine.
- Safety laboratory test results outside of normal institution reference ranges and considered clinically significant, classified by severity grading scale (irrespective of whether assessed as AEs).
- Any new or updated AEs that have met the holding rules.

The SMC will review the safety data within 2 working days. The SMC will summarize their recommendations to the study sponsor as to whether they approve of the investigator and sponsor's decision on further dose escalation and adjuvant use in groups 4 and above, whether there are safety concerns and whether the study should continue without change, be modified,

or be terminated. If at any time a decision is made to permanently discontinue administration of ABNCoV2 administrations in all subjects, the sponsor will notify the CCMO expeditiously.

9.5.4 Dose escalation and adjuvant use in group 4 and above

Further dose escalation and choice of adjuvant use in group 4 and above require approval by the SMC following review of all available safety, tolerability and immunogenicity data up to 14 days after first immunisation in all subjects up to and including group 3. The decision will be taken by the sponsor and the investigators and approved by a safety monitoring board based on all immunogenicity, safety and reactogenicity data that have been accrued until this time. The data to be reviewed includes all of the following available safety data:

- Solicited AEs through day 7 post first ABNCoV2 administration for all subjects in groups 1-3
- Unsolicited AEs through day 14 post first ABNCoV2 administration for all subjects in groups 1-3
- Clinically significant laboratory tests collected at 1 and 7 days following first ABNCoV2 administration for all subjects in groups 1-3
- Solicited and unsolicited AEs through day 7 post second ABNCoV2 administration for subjects in group 1A and sentinel subjects in group 1B and 2A.

The ABNCoV2 dose will be escalated until:

- The maximal dose of 70 µg is reached, or
- The vaccine is too reactogenic and/or not tolerated, or
- Immunogenicity-dose response curve is saturated (no further increase or decrease in antibody concentration with increasing dose)

The pre-defined criteria to decide on the optimal dose and formulation are:

- Safety and tolerability concerns during initial dose escalation of groups 1-3.
- Occurrence of related grade 3, serious and severe adverse reactions
- Dose response and adjuvant-dependency of anti-SARS-CoV-2 antibody titre

The SMC will review the AEs (local, systemic, and laboratory) and subsequently make their recommendation(s).

9.5.5 Holding rules for dose escalation to groups 4-7:

Decision on proceeding to group 4 will depend on the positive review by the SMC of the safety data containing solicited AEs through day 7 and unsolicited AEs through day 14 post first ABNCoV2 administration and clinically significant laboratory tests collected at 1 and 7

days following first ABNCoV2 administration for all subjects in groups 1-3. If either of the subjects in group 1-3 meet a holding rule (see below), administration of ABNCoV2 will be held for all remaining subjects in subsequent dose groups. The following holding rules apply to subjects in groups 1-3:

- One or more participants experience a SAE that is determined to be at least possibly related to the administration of ABNCoV2
- Two or more subjects experience a grade 3 adverse event (local, systemic or laboratory) possibly, probably or definitely related to ABNCoV2 administration and persisting at grade 3 for >48 hours

The study site member first aware of the event meeting the holding rule will notify the Principal Investigator and the Local Safety Monitor. The PI will alert the appropriate parties. The SMC will be notified within 24 hours. An ad hoc SMC review will be performed. The following considerations must be discussed:

- Relationship of the AE or SAE to the study product
- Relationship of the AE or SAE to ABNCoV2 dose
- If appropriate, additional screening or laboratory testing is provided to other subjects to identify subjects who may develop similar symptoms
- If any study related SAE is not listed on the current informed consent form (ICF), the PI will revise the ICF and subjects will be asked to provide consent on the new ICF

ABNCoV2 administration to subjects within the affected group and to the next higher dosage group may resume only if the local safety monitor, PI, SMC and the sponsor agree it is safe to resume ABNCoV2 administration. If the accredited METC has recommended safety hold, re-initiation of the study will require METC concurrence. The accredited METC will be informed of a safety hold by the sponsor. Following discussion, it may be decided to terminate the study.

All subjects who have received the study product will be followed for safety until resolution or stabilization (if determined to be chronic sequelae) of their AE(s). If at any time a decision is made to discontinue administration of study product in all subjects, expeditious notification will be provided by the Sponsor to the METC within 48 hours. The PI, local safety monitor, SMC or METC may stop or suspend the use of this product at any time.

9.5.6 Decision on further development of ABNCoV2

ABNCoV2 development will not be continued in case that no dosage and regimen shows acceptable safety and tolerability. Immunogenicity following first immunization (D14) of the optimized regimen shall be at least within the range of convalescent sera.

10. STATISTICAL ANALYSIS

COUGH-1 is an exploratory Phase 1 study without confirmatory aim. It is designed to inform dose, formulation and schedule for a larger Phase 2 clinical trial that will extent into higher age-groups – the most important risk group. Safety and tolerability data will be listed, immunogenicity data will be presented graphically and as aggregated data. A statistical analysis plan will be developed and finalized before first vaccination of a study participant.

Demographic and safety data will be presented as descriptive data without hypothesis testing. Immunogenicity data will be modelled using a Bayesian approach. This allows repeated estimation of the parameters following completion of each group and booster vaccination. Since the number of volunteers is small and the follow up is not complex, we do not expect much missing data. Nevertheless, we will perform sensitivity analyses to assess the effect of handling missing values, values below the lower limit of detection, different priors, models and distributional assumptions.

All analyses will be coded using R and follow guidelines for reproducible research.

10.1 Primary study endpoints

 # Number of at least possibly related Grade 3 AE and SAE from time of first administration of ABNCoV2 to the end of the follow-up period.

The total number of at least possibly related Grade 3 and SAE will be tabulated together with their severity grade (3 or serious) and relation to the vaccine (possible, probable or definitely related). Separate tables will be given for single groups, dosage and as number of volunteers experiencing AE.

All SAE will be described in from of short clinical summaries.

 # Concentration of ABNCoV2-specific antibodies 14 days following first vaccination.

ABNCoV2-specific antibodies will be measured by enzyme-linked immunosorbent assay (ELISA). Data will be transformed logarithmically to the base of 10 before aggregation or modelling. Data from pre-clinical assessment as well as published data show that log-transformation leads to a distribution for which the normal assumption can be assumed. Data below the lower limit of detection (i.e. most baseline data) will be imputed using half the mean of the lower limit of detection and the standard deviation.

10.2 Secondary study endpoint

 # Number and severity of at least possibly related solicited AEs within one week following administration of ABNCoV2 (day 0 to 7).

Here, the full AE tables will be given using the same tabulation method as for the primary endpoint. Severity will be reported on a 4-level ordinal scale: Grade 1, 2, 3, serious, causal relationship on a 5-level ordinal scale: not, unlikely, possible, probable, definitely related.

Since full AE tables are typically not easy to interpret (due to their size) and are a poor representation of AE patterns (e.g. interactions and co-occurrences) AE-networks will be displayed using igraph.

10.3 Other study endpoints

Other study endpoints are considered exploratory and hypothesis generating.

 # Concentration of ABNCoV2-specific antibodies at baseline and during immunization and follow up.

The kinetics of ABNCoV2-specific will be described using area under the curve, peak concentration and decay kinetics following first and second vaccination.

 # Inhibitory titre in invasion inhibition assay at baseline and during immunization and follow up.

The kinetics of functional activity of sera (by invasion inhibition assay) will be described using area under the curve, peak concentration and decay kinetics following first and second vaccination.

 # Cellular immune responses (T and B cell) at baseline and during immunization and follow up.

Cellular responses will be analysed by cytometry and enzyme-linked absorbent spot (ELISpot) assay.

10.4 Interim analysis (if applicable)

No interim analysis as in a confirmatory study setting is performed since a Bayesian approach to statistical calculation is used. Following first vaccination of Group 3B the database will be cleaned, AE data codes (using the medical dictionary for regulatory activities: MedDRA) and the immunogenicity data will be completed and validated. An interim report will be generated to guide the decision of investigator and sponsor to use adjuvanted or non-adjuvanted ABNCoV2 and to further dose-escalate. The decision process will be documented and presented, together with the data, to the SMC for approval.

The trial will be put on hold when a SAE at least possibly related to the vaccine occurs.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted in accordance with the latest Fortaleza revision of the Declaration of Helsinki (2013), the Medical research Involving Human Subjects act (WMO), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP), and local regulatory requirements. The investigators are responsible for obtaining all relevant ethical approvals of the protocol and any subsequent amendments in compliance with local law before the start of the study.

11.2 Recruitment and consent

As soon as the study is approved by the accredited METC and competent authority, healthy subjects will be recruited to participate in the study. Advertisements will be placed in prominent places on university campuses and other public places as well as on the intranet of the University and social media. The advertisement will indicate a telephone number to call and an e-mail address for contact to request further information. When seemingly suitable subjects contact investigators via e-mail, telephone or the online form, they will be invited to a (digital) information meeting during which the study will be explained to them by the study physician. Directly after the meeting they will be provided with documents to review at home (the test subject information, the informed consent form, the application form and the insurance text). During and after the meeting there will be time for questions. After this free discussion with the study physician, and any follow-up discussion if necessary, the volunteer will be given sufficient time to consider participation. Subjects who are interested in participating will be asked to fill in the application form and will be invited to come for a screening visit. Eligible subjects may only be included in the study after providing written, METC-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol, including screening procedures). The process of obtaining informed consent should be documented in the subject source documents. During the screening visit, the questionnaire answers will be discussed and inclusion and exclusion criteria will be checked. Again, the study physician will answer any questions the volunteer has. The possibility of withdrawal from the study, at any time, without penalty and without any declaration of the reason will be pointed out to the subjects. The investigators will be responsible for providing adequate verbal and written information regarding the objectives and procedures of the study, the potential risks involved and the obligations of the subjects. Subjects will be informed that they will not gain health benefits from this study. Trainees or other students who might be dependent on the investigators or the study group will not be included in the study.

11.3 Benefits and risks assessment, group relatedness

The compelling need for COVID-19 vaccines needs to be balanced with the potential risks and discomforts for the subjects. Risks for subjects are related to administration with ABNCoV2. There are no direct benefits to participation in the trial for subjects. Subjects will be advised to follow all the current national guidelines to minimize the risk of SARS-CoV2 transmission. All partners in this proposal are aware of and follow the relevant national and international rules and regulations as they pertain to access to material of human origin and clinical research. International agreements such as the Declaration of Helsinki will be observed and respected

11.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). The insurance offers a maximum coverage of:

1. € 650.000,-- (i.e. six hundred and fifty thousand euro) for death or injury for each subject who participates in the research;
2. € 5.000.000,-- (i.e. five million euro) for death or injury for all subjects who participate in the research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

This insurance provides cover for damage to research subjects through injury or death caused by the study.

11.5 Compensation

Enrolled subjects will receive up 1000,- euros in compensation for their time and for the inconveniences of taking part in this study. These amounts are based on predefined criteria:

- Screening and inclusion: 100,- euros
- Inconvenience of blood tests and/or visits: 25,- euros per blood sampling
- 2 x 1 day admission to hospital: 125,- euros per admission day
- ABNCoV2 administration: 300,- euros
- Compensation length of study: 20,- euros per month

Travel expenses will be additionally reimbursed by €0,19 per km, provided that the participant is travelling to the Radboudumc by car or public transport and lives more than 5 km from the Radboudumc. Compensation will not be provided to subjects who are not enrolled i.e. screen failures. Eligible subjects who are enrolled at the inclusion visit as back-ups, will be compensated 50,- euros for each inclusion visit. If a subject withdraws from the study prior to

completion, they will receive reimbursement proportional to number of visits they attended. These compensation amounts are reasonable and in line with Dutch common practice. In case of unexpected medical complications, there will be access to state-of-the-art medical treatment with full costs covered by the insurance of Radboudumc.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

A data management plan will be developed prior to start of study describing data management activities from project set-up through data lock and transfer. Designated trial staff will enter the data required by the protocol into the eCRF. All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification. An external monitor will review the data entered into the eCRFs by investigational staff for completeness and accuracy and will instruct the site personnel to make any required corrections or additions. Queries are made during each monitoring visit. Designated investigator site staff are required to respond to the queries and confirm or correct the data. Medical history/current medical conditions be coded using the ICD-10 terminology. AE will be coded using MedDRA.

12.1.1 Source data

Prior to the start of the trial it will be determined which documents completed by the investigative team will be considered source documents. Only authorized study staff and representatives of the sponsor and authorized regulatory agencies may have direct access to source documents containing study data. Data for this study will include biographical, medical history, clinical (signs, symptoms, prescription and non-prescription medical treatments), safety and laboratory data. All information in original records and certified copies of original records, clinical findings, or observations will be considered source data for this study. The memory aids, produced by the study subjects are also considered source data. They will be kept as source documents in the investigator's clinical file. Since all subjects will be healthy, there is no medical file for the study subjects, with exception of the medical file in case of adverse events/reactions resulting in a medical consultation or hospitalization. In this case the medical file will also be considered as the source data.

All data collected by the investigators is reported in the eCRF. All information in eCRFs must be traceable to the source documents in the subject's file. As with all parts of the eCRF, there is an audit trail in place to register every data entry. The investigator will also keep the original informed consent form signed by the subject (a signed copy is given to the subject). The investigator's team will maintain the information collected from CRFs and in

the eCRFs and all source documents that support the data collected from each subject in a secure area and treated as confidential material. Documents and data pertaining to the study will be kept in a locked cabinet under the responsibility of the investigator. Periodic monitoring visits will ensure that the data is safe and stored in a secure place and that only those authorized study staff have access to the data.

Source data from this study will be stored for 25 years, in line with the Nederlandse Federatie van Universitair Medische Centra (NFU) guideline. Biological samples from this study will be stored for maximum 15 years for research related to SARS-CoV2 immunology. New immunological or molecular tests may become available in the future that could strengthen or validate this research or help find important new findings. Should material be used for research not related to SARS-CoV2, permission from the METC must be granted.

12.1.2 Confidentiality

All parties agree to adhere to the principles of medical confidentiality in relation to clinical study subjects involved in this trial and shall not disclose the identity of subjects to third parties without prior written consent of the subject.

All data will be pseudonymised; volunteer data will be identified by a unique study number in the eCRF. Separate confidential files containing identifiable information will be stored in secured locations. All plasma samples, or other biological samples, with exception of those taken for safety diagnostics, will be labelled with the volunteer study identification number. Samples taken for safety diagnostic (processed by the central clinical laboratory) will be labelled with part of the subject identification code, study identification name and a fictitious birth date (only using the subjects actual birth year). The samples will not be labelled with volunteer names or actual birth dates. The subject identification code will be kept by the investigator.

The investigator will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH GCP Guidelines, any regulatory requirements, and institutional requirements for the length of storage and for the protection of confidentiality of subjects. The investigator will permit direct access to study records and source documents to authorized representatives of the sponsor, responsible ethics committee(s), regulatory agencies, and the external monitor(s), for the purposes of quality assurance reviews, audits / inspections, and evaluation of the study safety and progress. Direct access includes examination, analysis, verification, and reproduction of de-identified records and reports that are important to the evaluation of the trial.

12.2 Monitoring and Quality Assurance

General considerations

Before study initiation, the protocol and eCRFs together with relevant SOPs will be reviewed by the sponsor, the investigators and their staff. During and after completion of the study, the data monitor will visit the site to check the completeness of records, the accuracy of entries on the eCRFs, the adherence to the protocol and to Good Clinical Practice and the progress of enrolment.

The investigator will maintain source documents for each subject in the study, consisting of case and visit notes containing demographic and medical information, laboratory data, subject's diaries, and the results of any other tests or assessments. All information on eCRFs must be traceable to these source documents in the subject's file. As with all parts of the eCRF, there is an audit trail in place to register every data entry. The investigator will also keep the original informed consent form signed by the subject (a signed copy is given to the subject).

The investigator will give the external monitor access to all relevant source documents to confirm their consistency with the eCRF entries. According to the NFU risk classification system, this clinical trial has been classified as high risk. The monitor will perform full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs. The recording of data that will be used for all primary and safety endpoints will be assessed for 100% of included subjects.

External monitoring

To ensure that the study is conducted in accordance with ICH GCP and regulatory requirements, monitoring responsibilities will be provided by a Contract Research Organization or equivalent (CRO). A site initiation visit will be conducted prior to beginning of the study, and monitoring will be conducted at initiation, during, and at closeout of the study. During the course of the study, monitors will verify compliance to the protocol; completeness, accuracy, and consistency of the data and study product accountability; and adherence to ICH GCP and applicable regulations. As needed and when appropriate, the monitors will also provide clarifications and additional training to help the site resolve issues identified during the monitoring visit. As appropriate and informed by risk assessment, remote centralized monitoring activities may be considered in place of or to supplement onsite monitoring. These may include analysis of data quality (e.g., missing or inconsistent data), identification of data trends not easily detected by onsite monitoring, and performance metrics (e.g. screening or withdrawal rates, eligibility violations, timeliness, and accuracy of data submission).

The extent and frequencies of the monitoring visits will be described in a separate monitoring plan developed prior to study initiation. The investigator will be notified in advance of scheduled monitoring visits. The monitors should have access to all trial related sites, subject medical records, study product accountability, and other study-related records needed to conduct monitoring activities. The CRO will share the findings of the monitoring visit, including any corrective actions, with the site investigator. The site PI and the monitors must cooperate to ensure that any problems detected in the course of these monitoring visits are resolved in a predefined timeframe. All queries must be resolved prior to database lock. Essential documents must be filed in the site study file on an ongoing basis and be available for review by the CRO.

Independent Auditing

Sponsor representatives may audit the study to ensure that study procedures and data collected comply with the protocol and applicable SOPs at the clinical site and that data are correct and complete. The site PIs will permit auditors to verify source data validation of the regularly monitored clinical study. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and ICH GCP guidelines and applicable regulatory requirements.

Regulatory agency auditing (inspection)

The site PIs must be aware that regulatory authorities may wish to inspect the site records to verify the validity and integrity of the study data and protection of human research subjects. The site PIs must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies.

12.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments (e.g. typing errors and administrative changes like changes in names, telephone numbers and other contact details of involved persons mentioned in the submitted

study documentation) will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

The study will be entered by the investigator into a primary registry of the International Clinical Trials Registry Platform (ICTRP) network of the WHO (e.g. clinicaltrials.gov). The final report will be prepared by the investigators at the Radboud university medical center. It will be signed by the project leader or the principal investigator. The investigators will publish the results of the study, preferably in a peer-reviewed journal.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The ABNCoV2 vaccine is a sub-unit vaccine using a non-infectious virus-like particle (VLP) platform. A stabilized version of the receptor binding domain (RBD) of SARS-CoV-2 Spike glycoprotein (S) is bound to an VLP, and through vaccination will be presented to the immune system in order to evoke a response that should result in protection from infection.

Virus like particles (VLP) that display antigen on their surface are a technology to increase immunogenicity of soluble protein subunit vaccines. Their size (20-200nm) allows direct

drainage into lymph nodes and it is optimal for uptake by antigen-presenting cells and cross presentation. Their highly repetitive surface structures also facilitate complement fixation and B cell receptor clustering, leading to the activation of the innate immune system, greater B cell activation, and ultimately increased antibody production [31]. Heterologous antigens display on VLPs can assume a similar immunogenicity as the underlying particle, making VLPs good antigen-presenting platforms to increase immune responses against otherwise poorly immunogenic antigens [31, 42]. Besides induction of better immune responses, memory and specificity of the response may also be improved. Since VLPs cannot replicate they can be safely used in immunocompromised and senescent populations, two populations at risk for severe COVID-19.

In ABNCoV2 a novel VLP technology that allows covalent, directional, high density binding of different proteins on the VLP surface is used. The VLP consist of the coat protein of AP205, a structural protein of a virus that does not infect eukaryotes (including humans). In pre-clinical models ABNCoV2 immunogenicity has been superior to the other platform technologies [43]. In addition, it is optimized to comply with the WHO target product profile for SARS-CoV-2 vaccines [28].

MF59 is an oil-in-water emulsion adjuvant comprised of squalene, polysorbate 80, and sorbitan trioleate and is marketed in amongst others the Netherlands in seasonal influenza vaccines (e.g., Fluad/Fluad Tetra). MF59-adjuvanted vaccines spare vaccine doses and enhance hemagglutination inhibiting antibodies against homologous and heterologous influenza virus strains. The mechanisms of MF59 include rapid induction of chemokines and inflammatory cytokines, recruitment of multiple immune cells, increased uric acid (a post-apoptotic danger signal) and benign apoptosis of certain innate immune cells after IM injection [36]. The adjuvant effects of MF59 on generating vaccine-specific isotype-switched Immunoglobulin G (IgG) antibodies, effector CD8 T cells, and protective immunity were retained (even in a CD4-deficient condition) by inducing an effective immune-competent microenvironment with various innate and APCs in a mouse model. CD4-independent adjuvant effects of MF59 might contribute to improving vaccine efficacy in children, the elderly, and immunocompromised patients as well as in healthy adults [36].

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Besides ABNCoV2, more than 200 vaccines against SARS-CoV-2 are under development [14]. First candidates have reached Phase 3 clinical trials and promising results have been published in the form of press releases [15-17] and interim analyses [18, 19]. Three studies have reached approximately 100 cases, which allows a reasonable estimate of clinical relevant

vaccine efficacy. Observed VE was 60-90%. All three trials used candidates based on S antigen as the immunogen and have observed significant immunogenicity following two vaccinations. Two use an RNA-vaccine-platform and one a recombinant adenovirus. Follow up times are still short (median of ~3 months) but vaccination campaigns based on emergency-use authorization have started in several countries. In addition, systemic reactogenicity was significant in all three studies.

A AP205 VLP has never yet been administered to humans, but the closely related Phage Qβ phage VLP has been tested in clinical trials against non-infectious conditions. The CYT006-AngQb Phage Qβ cVLP vaccine targeting angiotensin 2 to reduce ambulatory blood pressure displayed an acceptable safety profile in phase 2 clinical trials (NCT00710372, NCT00500786, and NCT00701649). While transient flu-like symptoms were commonly observed, the most common AE were local injection site reactions. The vaccine caused a greater decrease in blood pressure when compared with a control group [25, 27].

A Phage Qβ cVLP has also been clinically evaluated as a vaccine against nicotine, intended for smoking cessation in phase 2 clinical trials (NCT00369616 and NCT01280968). For this vaccine, the most common AEs were transient flu-like symptoms followed by pyrexia, headache, nasopharyngitis, chills, and myalgia. For local reactions, injection site pain was the most frequently experienced AE, while injection site swelling, erythema, and edema were rarely experienced. The vaccine was found highly immunogenic and induced a mild attenuation of smoking behavior [26, 44].

The Phage Qβ cVLP has also been utilized as a toll-like receptor 9 (TLR 9) agonist to improve clinical outcome parameters of patients with allergic conditions in phase 1/2 clinical trials (NCT 00890734, NCT00800332, NCT00575003, NCT00574223, NCT00293904, NCT00574704, NCT00652223). In a trial examining patients with persistent allergic asthma, the most common AEs were injection site erythema, pruritus, and swelling and were primarily mild in intensity. Nasopharyngitis was also commonly reported, but not unexpected in this patient population. Of 33 patients who received cVLP treatment, 1 patient experienced moderate flu-like symptoms nearly a week after the third injection [45]. Rhino-conjunctivitis symptoms were significantly lower in patients treated with high dose of the cVLP TLR-9 agonist as compared with placebo [46].

Overall cVLP treatments have been shown to be generally safe and well tolerated in humans, with reports of mild flu-like symptoms and common vaccine-related injection site and systemic reactions being most common.

MF59 adjuvant is an oil-in-water emulsion marketed in the Netherlands as part of the influenza vaccine Fluad/Fluad Tetra. It is approved for use in the elderly (>65 years). The most common

injection-site reactions with MF59-adjuvanted Fluad influenza vaccines are injection site pain and tenderness [47]. The most common systemic reactions are myalgia, headache, and fatigue. In a clinical trial assessing elderly subjects aged 65 years or older (NCT02587221), injection site pain and influenza-like illness were the only unsolicited adverse reactions reported in ≥ 1% of subjects (1.7% and 1.5%, respectively) following vaccination with Fluad Quadrivalent. In another study (NCT03314662) injection site bruising was the only related unsolicited adverse reaction reported in ≥ 1% of subjects (1.0%) [48]. There were no SAE, AE of special interest, or deaths in either study that were related to study vaccine.

Overall MF59 adjuvanted influence vaccines have been shown to be generally safe and well tolerated in humans, with reports of common vaccine-related injection site and systemic reactions being most common.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

The ABNCoV2 vaccine induced CoV-2 spike protein-specific IgG in BALB/c mice after a single IM vaccination and these were boosted following a second dose. There was moreover significant correlation between ELISA antibody titers and neutralizing antibodies (Ks = 0.7152, p = 0.0461) in mice immunized with ABNCoV2. A single vaccination with ABNCoV2 resulted in 100% neutralization of SARS-CoV-2 at a serum dilution of 1:80, and serum post booster immunization could be diluted up to 1:10,240. It has been suggested that a >50% virus neutralization at an endpoint titer dilution of 100 to 500 would be required to confer protection [49]. Indeed, serum from mice immunized only once with ABNCoV2 displayed comparable neutralizing activity to high titer convalescent samples from patients recovered from COVID-19, whereas mice sera after multiple immunizations showed significantly greater virus neutralization activity than any human convalescent sera [43].

d. Selectivity of the mechanism to target tissue in animals and/or human beings

A repeat-dose toxicity study of ABNCoV2 with MF59 adjuvant in a 1:1 emulsion, vs. control (saline), administered IM 2 or 3 times (14 days apart) to New Zealand White (NZW) rabbits to assess toxicity and local tolerance showed no adverse risk profile.

The AP205 VLP has previously also been tested in non-human primates as a platform for delivering the malaria antigen, VAR2CSA. Overall, these vaccines were well-tolerated by the 40 aotus monkeys in this study, with no visible side effects, signs of systematic reactions, or behavioral changes. Additionally, the AP205 cVLP particle has been administered to hundreds of mice as well as rabbits, rats, and aotus monkeys in different vaccine formulations. In all

these studies, no issues were observed with weight loss, ulceration, or any other overt toxicities.

e. Analysis of potential effect

The ABNCoV2 dose administered in the rabbit toxicity study (0.1 mg/rabbit) translates to a human equivalent dose of >700 µg for a 60 kg human, providing, a safety margin of >7x higher than the highest dose envisaged to be administered in this FIH trial (70 µg).

In the aforementioned BALB/c mice study [43], the higher dose of 6.5 µg ABNCoV2 administered corresponds to a 10 to 20 times higher human equivalent dose (on a body surface area equivalence basis) than the highest dose to be tested in this trial.

f. Pharmacokinetic considerations

Biodistribution studies of ABNCoV2 have not been conducted because the vaccine does not include live virus and is not capable of replication or productive infection. The route of administration (IM) is well understood, and the vaccine will be formulated in MF59, a licensed adjuvant with previously defined characteristics. Consistent with International Council for Harmonisation (ICH) guidelines, formal pharmacokinetic studies were not conducted.

g. Study population

Subjects included in this trial are healthy adult volunteers, who have been extensively screened for any evidence of co-morbidity, including but not limited to: immune deficiency, hypersensitivity and cardiovascular risk factors. Female subjects of child-bearing potential are screened for pregnancy by urine test and are required to use contraception throughout the study period.

h. Interaction with other products

ABNCoV2 is not known to interact or be incompatible with any other products.

i. Predictability of effect

ABNCoV2-specific antibody concentrations will be measured by ELISA during immunisation and follow-up. Inhibitory titres will be measured in an *in vitro* SARS-CoV-2 invasion inhibition assay. In mice immunized with ABNCoV2 a significant correlation was observed between ELISA antibody titers and neutralizing antibodies (Ks = 0.7152, p = 0.0461). It has been predicted that a >50% virus neutralization at an endpoint titer dilution of 100 to 500 would be required to confer protection [49].

j. Can effects be managed?

Potential mild-moderate local or systemic adverse reactions to immunization will be managed symptomatically (e.g. paracetamol, ibuprofen, antihistamines). In the event of an anaphylactic reaction, this will be managed according to hospital-wide emergency protocols.

13.2 Synthesis

Preclinical studies show excellent immunogenicity of ABNCoV2, including high titres of neutralising antibodies, which are considered to represent a correlate of protection against SARS-CoV-2 infection and COVID-19. This is a first-in-human study and although pre-clinical toxicity results of ABNCoV2 are pending, substantive pre-clinical safety data exists on similar vaccines based on AP205 VLP, including in non-human primates. In addition, the closely related Phage Qβ phage VLP has been shown to be generally safe and well tolerated in humans, with reports of mild flu-like symptoms and common vaccine-related injection site and systemic reactions being most common.

The trial incorporates a number of safety designs to help minimize these and other residual risks: 1) Cautious dose-escalation, starting at 6µg (equating to ~140-400x below the human equivalent dose used in pre-clinical animal studies) and escalating 2-fold; 2) Administration of a give dose of the vaccine to a groups of volunteers first without MF59 adjuvant, before proceeding to administration of that dose to a separate group in combination with adjuvant; 3) Use of sentinel subjects within each dose-adjuvant group; 4) Review of safety data up to and including group 3 (25 µg with and without adjuvant) and approval by SMC required prior to further dose-escalation and decision on whether to include adjuvant in subsequent groups; 5) Repeat of highest and second-highest safe and tolerable doses in two additional groups of volunteers in order to further support confidence in safety data before proceeding to phase 2 trial(s).

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