Statistical Analysis Plan

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

Date: 25 April 2021

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1 Study details

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

Study code
COUGH-1

EudraCT number
2020-004621-22

Netherlands Trial Register Identifier
NL9334Version
1

Date
25 April 2021

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Table 1.1: Abbreviations

Abbreviation	Full form
AE	Adverse Event
AIC	Akaike information criterion
AR	Adverse reaction
CCMO	Centrale Commissie Mensgebonden Onderzoek
CoV	Coronavirus
Covid-19	Coronavirus disease 2019
CRF	Case Report Form
CTP	Clinical trial protocol
GCP	Good clinical practice
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IgG	Immunoglobulin G
ITT	Intention to Treat (Population)
MedDRA	Medical Dictionary for Regulatory Activities
NHP	Non-human primate
NPC	Non-pharmaceutical intervention
PCR	Polymerase Chain Reaction
PP	Per Protocol (Population)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS	Severe Acute Respiratory Syndrome
SD	Standard deviation
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
WAIC	Widely applicable information criterion
WHO	World Health Organization

2 Study design

2.1 Introduction

The Statistical Analysis Plan (SAP) describes the descriptive and inferential statistical analyses of tolerability, safety and immunogenicity data obtained in the “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" study. It is a controlled document and will be updated until the final analysis is performed. COUGH-1 is a Phase 1, open-labeled and exploratory clinical study on ABNCoV2, a vaccine to prevent infection with severe acute respiratory syndrome (SARS) Coronavirus (CoV) 2 and thereby avert coronavirus diseases 2019 (Covid-19).

2.2 Study rationale

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. Together with non-pharmaceutical interventions (NPC) a vaccine is a powerful tool 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 Covid-19 and limit spread of SARS-CoV-2.

2.3 Design

COUGH-1 is a phase 1, single center, 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. The vaccine will be formulated either without adjuvant or with the adjuvant MF59.

Data analysis is considered exploratory. The trial is designed to inform dosage and formulation for subsequent exploratory studies in groups at risk and bridging into confirmatory trials. It intends to inform dosage and formulation for subsequent clinical development.

2.4 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[1]. 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 (Part 1). Approval for further dose escalation and the further use of the adjuvant (MF59) 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-

[1] Originally, 100 µg were targeted as maximum dose. It was reduced to 70 µg due to CCMO request because of variablity in protein concentration in pre-clinical toxicological studies (no major signs of toxicity were observed).

vaccination. Recruitment for the two best (safe, tolerable and most immunogenic) regimens will continue until 12 volunteers per regimen have been immunized (Part 2).

3 Sample size considerations

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 and up to 7 reserve subjects will be recruited (1 reserve subject per dosage group) as replacements in case that a volunteer is not eligible on the first day of vaccination.

3.1 Safety

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 3.1 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 5.3% of the volunteers (within the tested dose-range), can be detected with 90% probability.

Table 3.1: Probability of Adverse Reactions

Sample size (n)	Probability of AR (%)	Power
6	31.9	0.9
12	17.5	0.9
18	12.0	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 adverse reactions 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.

3.2 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 during further dose-escalation 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).

Statistical analyses for immunological data will be developed based on assumptions derived from data generated during the discovery and pre-clinical stages of the project.

Assuming a dose-response relationship and an adjuvant effect similar to an ongoing non-human primate (NHP) study (Figure 3.1), a simulation using a linear model with dose and adjuvant as independent variables, i.e.

$$log10(IgG14_i) = \beta_0 + \beta_1 dose_i + \beta_2 adjuvant_i + \epsilon_i,$$

can inform on the power of the trial to detect an effect of the adjuvant 14 days following first immunization during the dose-escalation and adjuvant-selection part.

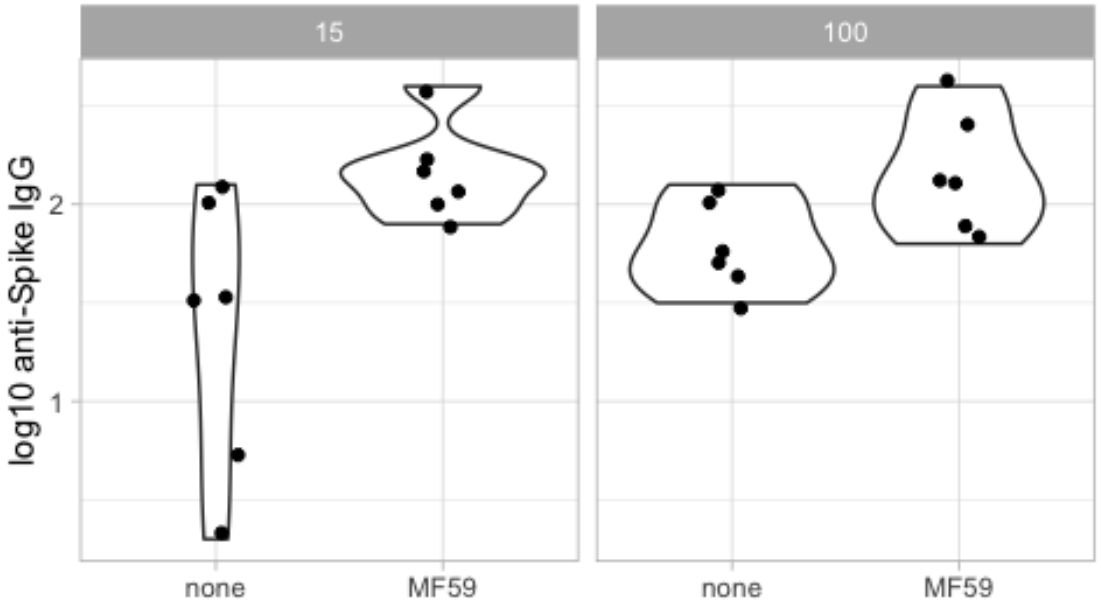

Figure 3.1: Anti-SARS-CoV-2 antibody titre following one inoculation of low (15 µg) and high (100 µg) dose ABNCoV2 in non-human primates. At baseline all values were below 1.

Based on the NHP study, we assume that the anti-S protein IgG concentration is log-normally distributed. Attained IgG concentration 14 days following first dose of 6, 12, and 25 µg of non-adjuvanted ABNCoV2 was set at 1, 1.25 and 1.5, respectively; following MF59-adjuvanted ABNCoV2 at 2, 2.25 and 2.5; a 10-fold higher concentration (of the non-transformed values) compared to non-adjuvanted ABNCoV2. The standard deviation (SD) was set at 0.7; the highest value found in the NHP study (low dose, non-adjuvanted; compare Figure 3.1; SD of the other conditions: 0.3), to get a conservative estimate of power. A simulated dataset of 30 participants per group is shown in Figure 3.2.

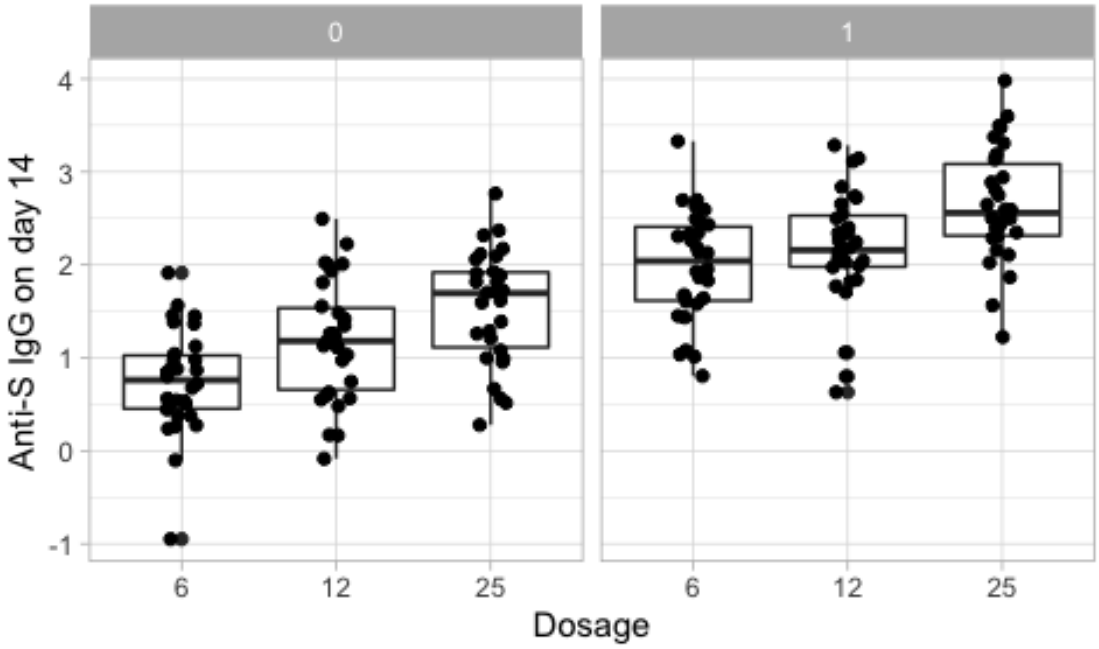

Figure 3.2: Simulated data for 30 participants per group.

Using 1000 simulations with a group size of three and $\alpha < 0.05$, shows approximately 80% power to detect a difference between the adjuvanted and non-adjuvanted groups and about 20% power to detect an effect of dose (Table 3.2). The main decision following the initial dose escalation is on the use of the adjuvant MF59 for further dose escalation. To assess the effect of dose, the second part of the study will recruit additional participants to increase power. Power will be recalculated before the second part of the trial based on the values observed in the trial.

Table 3.2: Power based on 1000 simulations (3 volunteers per group)

adjuvant	dose
0.808	0.185

4 Randomization

Allocation to dosage and formulation will not be randomized. Inclusion of volunteers to the different arms will be sequential.

4.1 Allocation concealment

Allocation to dose and formulation will not be concealed.

5 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.

6 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.

Secondary safety endpoint

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

Exploratory 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).

7 Study population

The study population is comprised of adult female and male healthy subjects aged 18-55 at time of first ABNCoV2 administration. A maximum of 42 subjects will be enrolled in the study. In addition, up to 7 reserve subjects (1 reserve subject per dosing group) will be recruited. The investigator will ensure that all subjects being considered for the study meet the eligibility criteria. 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.

7.1 Intention to treat population

The intention to treat population (ITT) is defined as all volunteers for whom at least one vaccination was formulated. Volunteers who received a partial vaccine injection or in whom violation of eligibility criteria were present (and detected posthoc) are part of the ITT population. Reserve volunteers are not part of the ITT population.

Modified ITT (mITT): All volunteers who received two vaccinations. This includes volunteers vaccinated out of the pre-specified time intervals (e.g. due to acute disease) and those with lacking visits.

7.2 Per protocol population

All volunteers who received all immunizations designated to the allocated group in the specified time intervals and adhered to the study procedures.

8 Data entry

Data are entered from Source data into an electronic data capture system (Castor) by the investigators and delegated personnel. Clinical source data are documented in the electronic medical record system at Radboudumc (Epic). Data will be monitored as described in the study's monitoring plan.

9 Statistical methods

The study is open labeled with at least one interim safety and immunogenicity analysis.

9.1 Demographics

Baseline characteristics (age, gender, height, weight, laboratory variables) will be tabulated. Categorical variables will be presented as count and percentage. Numerical variables will be summarized as median, minimum and maximum.

9.2 Study flow

A study flow chart (CONSORT flow chart) will be used to present number of volunteers screened, immunized and followed until completion of the trial.

9.3 Safety

Safety and tolerability data are presented as descriptive analysis, as listing and graphically. Adverse events (AE) will be recorded from the day of first vaccination until the end of the trial. Safety data of the ITT population will be presented. Verbatim-recorded AEs will be coded using MedDRA (Medical Dictionary for Regulatory Activities) and the proportion of

subjects with grade 3 AE and SAE classified by MedDRA preferred term level, will be tabulated.

9.4 Grading and causality assessment

AE grading — AEs are graded as Grade 1, 2, or 3. Where applicable, using predefined grading schemes (laboratory abnormalities and solicited AE).

Relationship to intervention — The clinical team using five pre-defined levels to assign causality to the study interventions: unrelated, unlikely related, possible, probable and definite relationship to the intervention. Where binary classification is required, 'unrelated' and 'unlikely related' will be grouped as Unrelated and 'possible', 'probable' and 'definite' will be grouped as Related.

9.5 Immunogenicity

Immunogenicity will be assessed by enzyme-linked immunosorbent assay (ELISA). Recombinant ExpreS2 produced SARS-CoV-2 Spike (35-1227) will be coated and antigen-specific immunoglobulin G (IgG) measured in three-fold serial dilution. Results will be reported as area under the curve (AUC) and endpoint titre.

Virus neutralization assay and antigen-specific cellular responses will be measured as part of exploratory analyses.

10 Conduct of analyses

10.1 Programming

All analyses will be programmed reproducibly using R and Stan (for Bayesian analyses). Interpretation and calculation of the data will use a Bayesian interpretation of probability and consequently not correct p-values because of repeated testing.

Demographic and safety data will be tabulated in summary tables as recommended in ICH Topic E 3.

The primary linear model for immunogenicity analyses 14 days following first vaccination (IgG14) will be

$$log10(IgG14_i) = \beta_0 + \beta_1 dose_i + \beta_2 adjuvant_i + \epsilon_i.$$

In addition, we will assess a model that takes into account baseline IgG concentration (IgG0):

$$log10(IgG14_i) = \beta_0 + \beta_1 log10(IgG0_i) + \beta_2 dose_i + \beta_3 adjuvant_i + \epsilon_i.$$

Models will be compared using information criteria (AIC, WAIC).

10.2 Coding

AE will be coded using MedDRA.

11 Document history

Version	Author	Description of change	Date
1	BMO	Not applicable	25 April 2021

12 Signature page

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