Protocol Number: CV-NCOV-001

Official Title: A Phase 1, partially blind, placebo-controlled, dose-escalation, first-in-human, clinical trial to evaluate the safety, reactogenicity and immunogenicity after 1 and 2 doses of the investigational SARS-CoV2 mRNA vaccine CVnCoV administered intramuscularly in healthy adults.

NCT Number: NCT04449276

Document Date: 25-Nov-2021

Statistical Analysis Plan (SAP)

Protocol Title:	A Phase 1, partially blind, placebo-controlled, dose-escalation, first-in-human, clinical trial to evaluate the safety, reactogenicity and immunogenicity after 1 and 2 doses of the investigational SARS-CoV-2 mRNA vaccine CVnCoV administered intramuscularly in healthy adults.
Protocol Version No./Date:	3.0/24-JUL-2020
CRF Version No./Date:	4.0/01-FEB-2021
SAP Version No./Date:	3.0/25-NOV-2021

1.0 Approvals

Sponsor	
Sponsor Name:	CureVac AG
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(NOTE: Electronic Signatures should only be used if all parties have the ability to eSign.)

2.0 Change History

Version/Date	Change Log
1.0/23-JUL-2020	Created as new
2.0/17-SEP-2020	Section 8.0 updated to reflect protocol version 3.0 Section 10.4.7 Adverse Reactions added – definition added Section 10.4.8 Credibility Interval and related Estimate - clarification regarding calculation added Section 10.5 Retrospective SARS-CoV-2 Baseline Serology – definition added Section 10.6 Subjects not exposed to SARS-CoV-2 before or during the trial – definition added Section 10.7.1 Geometric mean – clarification regarding calculation added Section 10.7.2 Seroconversion – definition added Section 11.1 Interim Analyses – details for scope added Section 12.6.3.1 Secondary Endpoints; clarification of analysis and fold change, mean fold change and box plots added for ELISA IgG/IgM and VNT. Appendix 3 Statistical details added Appendix 4 Inclusion/Exclusion criteria updated
3.0/25-NOV-2021	Section 10.5.4 Evaluation of infection - definition added Section 10.6 Censoring rule for subjects unblinded due to availability of authorized/licensed vaccine Section 12.2 Important protocol deviations – process definition added Section 12.6.2 Adding analysis of occurrence of AEs related to SMQs. Section 12.6.3.1 Additional analysis for subjects receiving both doses, are seronegative and did not have any protocol deviations with impact to immune response

3.0 Table of Contents

4.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under CureVac AG Protocol CV-NCOV-001.

5.0 Scope

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Endpoints and Assessments
- Applicable Study Definitions
- Analysis Sets
- Statistical Methods
- Conventions and Definitions

6.0 Introduction

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under CureVac AG Protocol CV-NCOV-001.

The SAP should be read in conjunction with the

- Study protocol version 3.0, 24-JUL-2020
- Case report form (CRF) version 3.0, 09-JUL-2020

Changes following approval of the first version of the SAP will be tracked in the SAP Change Log and a final version of the amended SAP will be approved prior to final database lock. Final approval of this document will occur prior to database lock.

Each version of the SAP requires approval by the Sponsor.

6.1 Changes from Protocol

No changes.

7.0 Study Objectives

All objectives will be analyzed in all subjects, in those subjects retrospectively SARS-CoV-2 seronegative at baseline, and in those subjects retrospectively SARS-CoV-2 seropositive at baseline.

7.1 Primary objectives

- To evaluate the safety and reactogenicity profile after 1 and 2 dose administrations of CVnCoV at different dose levels.

7.2 Secondary objective

- To evaluate the humoral immune response after 1 and 2 dose administrations of CVnCoV at different dose levels.

7.3 Exploratory objectives

- To evaluate the cell-mediated immune response after 1 and 2 dose administrations of CVnCoV at different dose levels in all subjects from the assigned site(s).

- To evaluate the innate immune response after 1 and 2 dose administrations of CVnCoV at different dose levels in all open-label sentinel subjects.
- To identify and assess cases of COVID-19 disease.
- To describe the rate of asymptomatic infections with SARS-CoV-2.

8.0 Study Design

8.1 Overall Design

This is a Phase 1, partially blind, placebo-controlled, dose escalation first-in-human (FIH) trial of intramuscularly (IM) administered CVnCoV. This trial will evaluate the safety, reactogenicity and immunogenicity of 3 provisional CVnCoV dose levels (2, 4 and 8µg) using an adaptive dose-finding design. This will allow dose escalation or de-escalation using predefined safety criteria and select the CVnCoV dose for further clinical development. To ensure the safety of the subjects, specified safety data will be reviewed on a predefined schedule by an independent safety review committee (iSRC) and a data safety monitoring board (DSMB).

An adaptive 2-parameter Bayesian logistic regression model (BLRM) for dose escalation with overdose control (EWOC) will be used in the escalation to guide determination of the safety of each dose level, while decisions of dose level expansion in the sentinel groups of 4 subjects is based on a maximum number of 2 subjects with Grade 3 adverse reactions.

Subjects will be enrolled in 2 age categories (18-40 years and 41-60 years) with an equal distribution across each category. The trial will include subjects with no history of COVID-19 disease as well as subjects with SARS-CoV-2 positive serology. The SARS-CoV-2 serology status at baseline will be evaluated retrospectively to allow separate analysis of subjects retrospectively SARS-CoV-2 seronegative at baseline and subjects retrospectively SARS-CoV-2 seropositive at baseline. To ensure the open-label sentinel subjects in each dose level are seronegative, the SARS-CoV-2 serological status will be determined prior to enrollment during eligibility assessment in these subjects. Additionally, assessment of SARS-CoV-2 serological status may also be performed in the observer-blind placebo-controlled part of the trial, to identify subjects with SARS-CoV-2 positive serology.

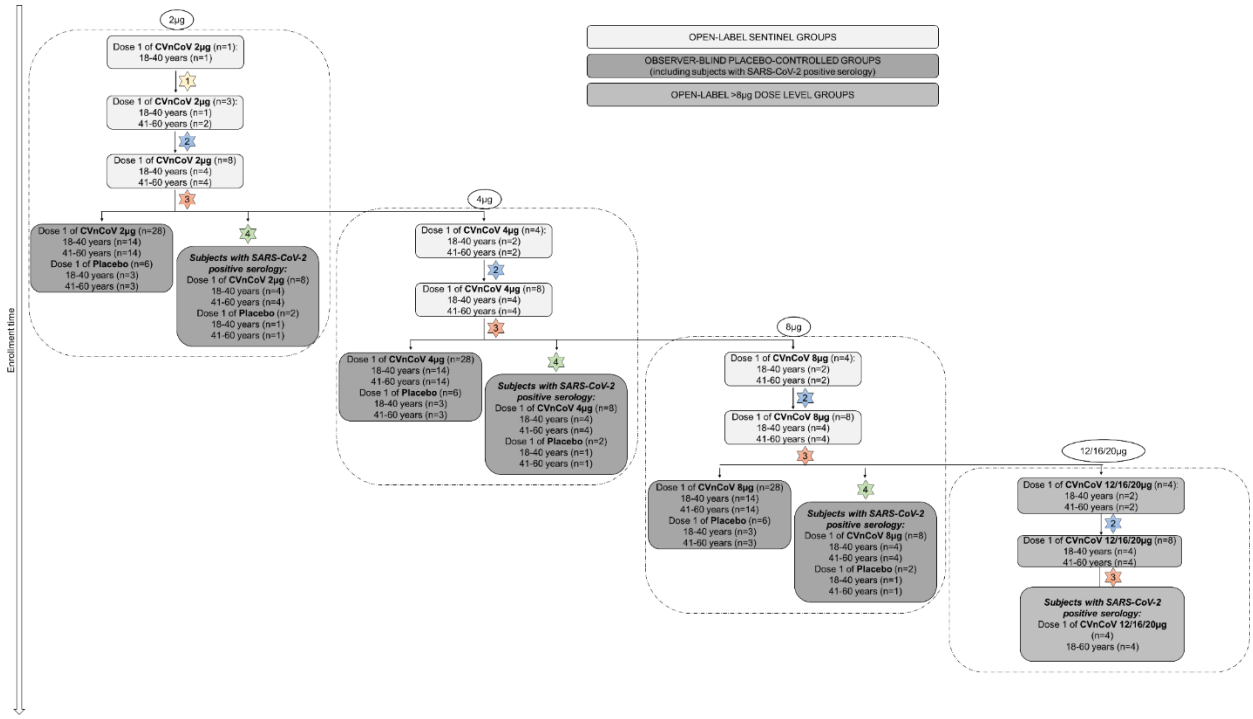
Due to the adaptive design of the trial, the actual number of subjects enrolled might be lower or higher than the target numbers.

Throughout the trial, cases of COVID-19 disease will be identified and documented for later pooling of cases across trials in the clinical development program.

Subjects will be enrolled in 4 trial sites, 3 in Germany and 1 in Belgium. The duration for each subject is approximately 13 months.

A schematic overview of the design is provided in Figure 1 below.

Figure 1 Overview of staggered enrollment during the dose escalation phase



The 12, 16 and/or 20µg dose levels will only be given to subjects once initial data from the previous dose level have been reviewed by the iSRC and DSMB, not in parallel.

8.1.1 Dose Escalation Steps

The starting dose of CVnCoV is 2µg. Dose escalation steps will follow the scheme indicated in Table 1. In each dose escalation step, subjects will be equally distributed across the 2 age categories (with the exception of subjects with SARS-CoV-2 positive serology who receive dose levels >8µg).
Additional potential dose levels that could be assessed are 1, 3 and 6µg, depending on the reactogenicity/safety findings after vaccination with respectively 2, 4 and 8µg dose levels; as well as 12, 16 and/or 20µg.

All subjects will be administered a second vaccine dose on Day 29 with the same dose level of CVnCoV or placebo as administered on Day 1. When safety data will be available at least 60 hours post dose 2 for all subjects within a dose level, the iSRC and DSMB will review all available safety (and, if available, immunogenicity) data to confirm the safety of the dose level after the second dose.

Table 1 Provisional dose levels during dose escalation

Number of subjects vaccinated per CVnCoV dose level (µg)*							
2µg		4µg		8µg		12/16/20µg****	
18-40 years	41-60 years	18-40 years	41-60 years	18-40 years	41-60 years	18-40 years	41-60 years
1	-	-	-	-	-		
Post-vaccination observation for 24 hours**		-	-	-	-		
1	2	-	-	-	-		
Safety review of 24-hour post dose 1 data (iSRC+DSMB Chair)		-	-	-	-		
4	4	-	-	-	-		
Safety review of 60-hour post dose 1 data (iSRC+DSMB)							
18 + 4 placebo***	18 + 4 placebo***	2	2	-	-		
-	-	*Safety review of 24-hour post dose 1 data (iSRC+DSMB Chair)*		-	-		
-	-	4	4	-	-		
Safety review of 60-hour post dose 1 data (iSRC+DSMB)							
-	-	18 + 4 placebo***	18 + 4 placebo***	2	2		
-	-	-	-	*Safety review of 24-hour post dose 1 data (iSRC+DSMB Chair)*			
-	-	-	-	4	4		
Safety review of 60-hour post dose 1 data (iSRC+DSMB)							
-	-	-	-	18 + 4 placebo***	18 + 4 placebo***	2	2
-	-	-	-	-	-	*Safety review of 24-hour post dose 1 data (iSRC+DSMB Chair)*	
-	-	-	-	-	-	4	4
Safety review of 60-hour post dose 1 data (iSRC+DSMB)							
-	-	-	-	-	-	**18 to 60 years**	
-	-	-	-	-	-	4*****	

Light grey shaded cells indicate the open-label sentinel groups.
Dark grey shaded cells indicate the observer-blind placebo-controlled groups.
* Provisional dose levels of 2, 4 and 8µg will be evaluated. Additional potential dose levels that could be assessed are 1, 3 and 6µg, depending on the reactogenicity/safety findings after vaccination with respectively 2, 4 and 8µg dose levels; as well as 12, 16 and/or 20µg.
** If any Grade 3 ARs are reported within 24 hours following vaccination, the iSRC and DSMB Chair will be consulted before continuation of enrollment of the next open-label sentinel subjects.
*** Including approximately 4 + 1 subjects with SARS-CoV-2 positive serology.
**** The next dose level will only be given to subjects once initial data from the previous dose level have been reviewed by the iSRC and DSMB, e.g., if 12µg is investigated, it will only be administered after review of the 8µg data (post dose 1 in 12 sentinel subjects), and if the dose level is then escalated to 16µg, it will only be administered after review of the 12µg data (post dose 1 in 12 sentinel subjects).
***** Subjects with SARS-CoV-2 positive serology.

On the first vaccination day, the first open-label sentinel subject aged 18-40 years confirmed to be SARS-CoV-2 seronegative will be enrolled and vaccinated with CVnCoV at the lowest dose level (2µg). The subject will be observed for 24 hours after vaccination. If any Grade 3 adverse reactions, defined as solicited systemic, solicited local and unsolicited Grade 3 adverse events (AEs) considered as related to the trial vaccine according to the Investigator, are reported within 24 hours following vaccination, the iSRC and DSMB Chair will be consulted. In case no safety concerns are identified, the next open-label sentinel group of 3 subjects (1 aged 18-40 years and 2 aged 41-60 years) confirmed to be SARS-CoV-2 seronegative will be enrolled and vaccinated with CVnCoV at the lowest dose level (2µg). The subjects should be vaccinated at least 60 minutes apart. Safety and reactogenicity data reported during an observation period of at least 24 hours after vaccination from these first 4 subjects will be collected and reviewed by the iSRC and DSMB Chair. In this review, the iSRC and DSMB Chair will review all available safety data, but focus specifically on Grade 3 adverse reactions, defined as solicited systemic, solicited local and unsolicited Grade 3 AEs considered as related to the trial vaccine according to the Investigator, and any SAE considered as related to the trial vaccine according to the Investigator. Based on this review, the iSRC and DSMB Chair will decide on the subsequent vaccination of an additional open-label sentinel group of 8 subjects (4 aged 18-40 years and 4 aged 41-60 years, confirmed to be SARS-CoV-2 seronegative) with the same dose level on the next vaccination day (at least 36 hours after the first vaccination day).

Safety and reactogenicity data of subjects vaccinated on the first and second vaccination days will then be collected for at least 60 hours post-vaccination from each subject and reviewed by the iSRC and the DSMB. Based on this review, the iSRC and DSMB will recommend on continuation of enrollment at the same dose level (start of randomized expansion in observer-blind placebo-controlled groups) and initiation of enrollment in the next dose level:

- In case of **continuation of enrollment at the 2µg dose level**, an additional 44 subjects will be enrolled, including 10 subjects with SARS-CoV-2 positive serology. Of these 44 subjects, 36 subjects (18 aged 18-40 years and 18 aged 41-60 years) will receive CVnCoV at the 2µg dose level, including approximately 8 subjects with SARS-CoV-2 positive serology. The other 8 subjects (4 aged 18-40 years and 4 aged 41-60 years) will receive placebo in an observer-blind manner, including approximately 2 subjects with SARS-CoV-2 positive serology.
- In case of **dose escalation**, a newly enrolled open-label sentinel group of 4 subjects (2 aged 18-40 years and 2 aged 41-60 years) confirmed to be SARS-CoV-2 seronegative will receive the next higher dose (4µg) or an intermediate dose depending on iSRC and DSMB recommendations guided by the BLRM. The subjects should be vaccinated at least 60 minutes apart. In case of favorable outcome of the review by the iSRC and DSMB Chair of at least 24-hour post-vaccination data and a maximum number of 2 subjects with Grade 3 adverse reactions, an additional open-label sentinel group of 8 subjects (4 aged 18-40 years and 4 aged 41-60 years, confirmed to be SARS-CoV-2 seronegative) will receive the same dose level on the next vaccination day (at least 36 hours after the first vaccination day).

For each subsequent step, continuation of enrollment within a dose level in a placebo-controlled observer-blind manner and dose escalation will be assessed by the iSRC and DSMB based on 60-hour post-vaccination safety data for each subject, including all available post-vaccination data from previously vaccinated subjects. The scheme for staggered enrollment and dose escalation in Figure 1 will be pursued if no safety concerns are found by the iSRC or DSMB.

Once the open-label sentinel phase has been successfully concluded for a given dose level, enrollment will no longer be restricted to subjects with no history of COVID-19 disease or negative serology to SARS-CoV-2, but will also include subjects with SARS-CoV-2 positive serology. These subjects will be enrolled at pre-defined site(s). For each dose level, the iSRC will review at least 24-hour post-vaccination safety data from the first 4 seropositive subjects. These first 4 seropositive subjects within each dose level should be vaccinated at least 60 minutes apart. Vaccination of additional subjects at that dose level and/or vaccination

of subjects with SARS-CoV-2 positive serology at a higher dose level will only proceed upon favorable outcome of this iSRC review.

8.2 Adaptations of the Trial

Depending on the safety and immunogenicity of the provisional dose levels, additional dose levels might be evaluated. In each case, the enrollment schedule will be performed as described as above.

For dose levels >8µg, the enrollment schedule will be performed as described in Section 8.1.1 for the first 12 sentinel subjects. After a review of the post dose 1 data from these subjects, 4 subjects (aged 18 to 60 years) with SARS-CoV-2 positive serology will be enrolled to receive the same CVnCoV dose level. These 4 open-label subjects will have the same assessments as subjects in the observer-blind placebo-controlled groups and are included when reference is made to the observer-blind placebo-controlled groups.
In case the lowest dose of 2µg fails to meet the predefined safety criteria, a dose de-escalation to 1µg will be initiated. In case the 4µg dose level fails to meet the predefined safety criteria, a dose de-escalation to 3µg can be initiated. Similarly, in case the 8µg dose level fails to meet the predefined safety criteria, a dose de-escalation to 6µg can be initiated.

8.2.1 Stopping Rules for Second Dose

Individual stopping AE rules will be applicable during the entire trial to ensure safe administration of the second dose to subjects vaccinated with the first vaccine dose.

The stopping rules are met in case any of the following events occur on the day of vaccination or following 7 days (Days 1-8):

- An allergic/anaphylactic reaction considered as related to the trial vaccine,
- Any SAE considered as related to the trial vaccine,
- Any Grade 3 AE considered as related to the trial vaccine with the following exceptions:
  - Transient Grade 3 systemic AE (fever, headache, fatigue, chills, myalgia, arthralgia, nausea/vomiting and diarrhea) considered as related to the trial vaccine that resolved within 48 hours to Grade ≤2,
  - Transient Grade 3 local AE that resolved within 48 hours to Grade ≤2.

If any of these rules are met, the subject must not receive the second vaccine dose. The subject will be encouraged to continue participation until the end of the trial for safety and immunogenicity assessments.

8.2.2 Trial Suspension Rule

A trial suspension rule will be applicable during the entire trial. This rule is met if a subject vaccinated with CVnCoV experiences an SAE considered as related to the trial vaccine by the Investigator or Sponsor.

If this rule is met, enrollment and vaccination with CVnCoV will be suspended within 24 hours of awareness by the trial team. An ad-hoc DSMB meeting will be held to review all safety data per the DSMB charter. Depending on the DSMB assessment of the benefit-risk ratio, including the relationship of the SAE to the trial vaccine, enrollment and vaccination with CVnCoV might be temporarily halted and only be re-started upon approval by the DSMB and competent authority.

During the observer-blind placebo-controlled phase at each dose level, if >4 subjects experienced Grade 3 adverse reaction(s) after administration of CVnCoV at a particular dose level, vaccination will be put on hold for all subjects in the applicable dose level and any higher dose level. The DSMB will perform a comprehensive review of all relevant safety and reactogenicity data before making a decision to stop, continue or modify vaccination (including dose-de-escalation) with this and any higher dose level.

8.2.3 Justification for Dose

Three provisional CVnCoV dose levels (2, 4 and 8 µg) will be evaluated, defined based on clinical data from the CV7202-104 trial with a Rabies vaccine using the same technology as well as non-clinical data. Refer to the Investigator's Brochure for an overview of these data.
Additional higher (12, 16 and/or 20µg), lower (1µg) and/or intermediate (3 and 6 µg) dose levels might be evaluated depending on the outcomes of the safety and reactogenicity evaluations and all available immunogenicity data. Identification of immunogenic dose levels with lower mRNA content might be of particular importance for efficient vaccination in a worldwide viral outbreak setting

8.2.4 End of Trial Definition

A subject is considered to have completed the trial when he/she has completed all visits applicable for the group to which he/she was randomized/assigned to at trial entry.

End of Trial is defined as the point at which the last subject has completed the last visit on Day 393.

8.3 Sample Size Considerations

This trial is designed to estimate the probability that the true rate of adverse reactions for each dose lies in an acceptable safety range (see Section 12.6) and to describe the safety profile of CVnCoV. Hence, its sample size was not determined based on formal statistical power calculations.

During the trial, a minimum of 12 subjects evaluable for the dose determining set (DDS-2, as defined in Section 9.5.3) will be vaccinated per dose level until completion of the dose escalation. After completion of the open-label sentinel phase, subjects will be enrolled in placebo-controlled groups in an observer-blind manner within each dose level with a 4.5:1 ratio and stratified by the 2 age categories (18-40 and 41-60 years).

It is estimated that approximately 56 subjects per dose level will be enrolled, including 48 CVnCoV and 8 placebo recipients, for ≤8µg and 16 subjects per dose level, all CVnCoV recipients, for >8µg. The actual number of subjects will depend on the number of dose levels/groups that are tested.

The trial is designed to provide, by combining data from the open-label sentinel and the observer-blind placebo-controlled phases, a reasonable precision for the rate of subjects with Grade 3 adverse reaction(s). Table 2 provides the estimate and 95% credibility interval for given observed numbers of subjects for a sample size of 48 subjects per dose level (assuming a Beta (0.5, 0.5) prior distribution).

Table 2 Estimate rate of subjects with Grade 3 adverse reaction(s) and 95% credibility interval per dose level assuming a Beta(0.5. 0.5) prior distribution

N subjects with Grade 3 adverse reaction(s)	Estimate (95% credibility interval) in %	Probability adverse reaction rate <33%
0	1 (0 - 5.1)	100
2	5.1 (0.9 - 12.7)	100
4	9.2 (2.9 - 18.6)	100
6	13.3 (5.4 - 24)	99.9
8	17.3 (8.2 - 29)	99.4
10	21.4 (11.2 - 33.8)	96.7
12	25.5 (14.5 - 38.5)	88.2
14	29.6 (17.8 - 43)	71.,0
16	33.7 (21.3 - 47.3)	47.3
18	37.8 (24.9 - 51.6)	25.1
20	41.8 (28.5 - 55.8)	10.2

N = Number of subjects

Table 3 provides probabilities (in %) that the following condition is met for assumed true rates of adverse reactions and a sample size of 48 subjects per dose level: There is a ≥80% probability that the true rate of Grade 3 adverse reaction(s) is <33%.

For example, assuming a true adverse reactions rate of 20%, the condition is met with a probability of 91.6%.

Table 3 Probabilities that the rate of Grade 3 adverse reactions is <33% is ≥80% for given 'true' adverse reactions rates during dose escalation for n=48 and assuming a Beta(0.5, 0.5) prior distribution

True rate of adverse reactions	Probability (%)
0.05	100
0.1	100
0.15	99.1
0.2	91.6
0.25	69.8
0.3	39.7
0.35	16.1

The number of placebo subjects is chosen to have a minimum number of subjects to descriptively evaluate the onset and the level of humoral immune response taking into account that there are 2 age groups, 2 possible SARS-CoV-2 status at baseline, 3 different doses levels and a placebo group (Table 4). A total of 24 placebo subjects, of whom 18 are expected to have a SARS-CoV-2 negative serology before the trial start, will provide a reasonable number in the placebo group to differentiate between each of the age groups receiving CVnCoV individually and all subjects on a dose level versus all placebo recipients. Despite the small sample size for the placebo group within a dose level, a pooled within-trial analysis of all placebo recipients could provide a sufficient number to assess differences between CVnCoV and placebo recipients by providing anchoring information on 'no dose' for regression analyses. With the given number, an exploratory t-test might be conducted assuming a large vaccination effect, which cannot be quantified yet. As the effect is unknown since this is the FIH trial, no assumptions and power analyses were conducted. This trial will however allow to generate a hypothesis for the Phase 2/3 trials.

Table 4 Distribution of subjects as per the planned dose levels

	Subjects with no history of COVID-19		Subjects with SARS-CoV-2 positive serology	
	CVnCoV	**Placebo**	**CVnCoV**	**Placebo**
2/4/8µg: 18-40 years	20	9	4	3
2/4/8µg: 41-60 years	20	9	4	3
2/4/8µg: all age groups	40	18	8	6

In addition, even in the absence of statistical power, the inclusion of blinding and placebo subjects can reduce biases in AE reporting occurring in open-label trials.

This trial is designed to estimate the probability that the true rate of adverse reactions for each dose lies in an acceptable safety range, and to describe the safety profile of CVnCoV. Hence, its sample size was not determined based on formal statistical power calculations.

During the trial, a minimum of 12 subjects evaluable for the dose determining set (DDS-2, as defined in Section 9.5.3) will be vaccinated per dose level until completion of the dose escalation. It is estimated that approximately 168 subjects (56 per dose level) will be enrolled, including 144 CVnCoV recipients (48 per dose level). The actual number of subjects will depend on the number of dose levels/groups that are tested. The trial is designed to provide, by combining data from the open-label sentinel and the observer-blind placebo-controlled phases, a reasonable precision for the rate of subjects with Grade 3 adverse reaction(s).

8.4 Randomization

In the initial part of dose escalation for the provisional dose levels of 2, 4 and 8µg, subjects will be enrolled in sentinel groups in an open manner. In the second part, subjects will be enrolled in placebo-controlled groups in an observer-blind manner within each dose level with a 4.5:1 ratio and stratified by the 2 age categories (18-40 and 41-60 years). Double-blinding is not possible due to the difference in appearance of the investigational vaccine and placebo.

Groups with a dose level >8µg will be open-label.

8.5 Blinding/Unblinding

The Sponsor and safety monitoring committees will be unblinded for data from the placebo-controlled groups, but will take appropriate measures to ensure subject blinding is kept at site-level until database lock.

9.0 Study Endpoints

9.1 Primary Endpoints

- The frequencies of Grade 3 adverse reactions and any SAE considered as related to the trial vaccine within at least 24 hours after the first vaccination by dose level, for decisions on subsequent vaccination of an additional open-label sentinel group with the same dose level.
- The frequencies of Grade 3 adverse reactions and any SAE considered as related to the trial vaccine within at least 60 hours after the first vaccination by dose level, for decisions on dose escalation as well as continuation of enrollment at the same dose level in the observer-blind placebo-controlled part of the trial.
- The frequencies, intensities and duration of solicited local AEs on each vaccination day and the following 7 days by dose and dose level, for the characterization of the safety and reactogenicity profile.

- The frequencies, intensities, duration and relationship to trial vaccination of solicited systemic AEs on each vaccination day and the following 7 days by dose and dose level, for the characterization of the safety and reactogenicity profile.
- The occurrence, intensities and relationship to trial vaccination of unsolicited AEs on each vaccination day and the following 28 days by dose and dose level, for the characterization of the safety and reactogenicity profile.
- The occurrence and relationship to trial vaccination of SAEs and AESIs at predefined time points throughout the trial, for the characterization of the safety and reactogenicity profile.

9.2 Secondary Endpoints

On Day 8, Day 15, Day 29, Day 36, Day 43, Day 57, Day 120, Day 211 and Day 393:

- The proportion of subjects seroconverting for SARS-CoV-2 spike protein antibodies, as measured by enzyme-linked immunosorbent assay (ELISA).
- Individual SARS-CoV-2 spike protein-specific antibody levels in serum, as measured by ELISA.
- Geometric mean titers (GMTs) of serum SARS-CoV-2 spike protein antibodies, as measured by ELISA, in subjects who did not get exposed to SARS-CoV-2 before the trial, or during the trial before the applicable sample was collected, as measured by ELISA to SARS-CoV-2 N-antigen.
- The proportion of subjects seroconverting for SARS-CoV-2 neutralizing antibodies, as measured by an activity assay.
- Individual SARS-CoV-2 neutralizing antibody levels in serum.
- GMTs of serum SARS-CoV-2 neutralizing antibodies, as measured by an activity assay, in subjects who did not get exposed to SARS-CoV-2 before the trial, or during the trial before the applicable sample was collected, as measured by ELISA to SARS-CoV-2 N-antigen.

9.3 Exploratory Endpoints

Cell-mediated immune response

*On Day 29, Day 36 and Day 211** in peripheral blood mononuclear cells (PBMCs) from all subjects at the assigned site(s):*

- The frequency and functionality of SARS-CoV-2 spike-specific T-cell response after antigen stimulation.

  Intracellular cytokine staining (ICS) to investigate Th1 response and production of Th2 markers (e.g. secreted interleukin [IL-5]) will be used to investigate whether vaccination induces a Th1 shift from baseline. Further T-cell immune response may be investigated with other technologies like ELISpot or CyTOF.
- The proportion of subjects with a detectable increase in SARS-CoV-2 spike-specific T-cell response.

*** Testing of samples collected on Day 211 will be done only in subjects categorized as T-cell responders on Day 29 and/or Day 36.*

Innate immune response

On Day 2, Day 8, Day 29, Day 30 and Day 36 in all open-label sentinel subjects:

- Serum cytokine concentrations, including but not limited to interferon (IFN)-α, IFN-γ, IL-6, chemokine ligand (CCL) 2 and IFN-γ-induced protein 10 (IP-10).
- Gene expression profiling.

** Samples may be used for assay validation and additional studies of the mechanism of action of the vaccine.*

Evaluation of infection

- Number of subjects with virologically-confirmed SARS-CoV-2 infection as measured by reverse transcription polymerase chain reaction (RT-PCR) at clinically determined time points throughout the trial.
- Number of subjects with asymptomatic SARS-CoV-2 infection as measured by retrospective serology at predefined time points.

9.4 Endpoint Attributes

Objectives	Endpoints	Population
Primary		
• To evaluate the safety and reactogenicity profile after 1 and 2 dose administrations of CVnCoV at different dose levels.	• The frequencies of Grade 3 adverse reactions and any SAE considered as related to the trial vaccine within at least 24 hours after the first vaccination. • The frequencies of Grade 3 adverse reactions and any SAE considered as related to the trial vaccine within at least 60 hours after the first vaccination. • The frequencies, intensities and duration of solicited local AEs on each vaccination day and the following 7 days. • The frequencies, intensities, duration and relationship to trial vaccination of solicited systemic AEs on each vaccination day and the following 7 days. • The occurrence, intensities and relationship to trial vaccination of unsolicited AEs on each vaccination day and the following 28 days. • The occurrence and relationship to trial vaccination of SAEs and AESIs throughout the trial.	DDS-1* DDS-2* Safety Set
Secondary		
• To evaluate the humoral immune response after 1 and 2 dose administrations of CVnCoV at different dose levels.	• Seroconversion for SARS-CoV-2 spike protein antibodies, as measured by ELISA. • Individual SARS-CoV-2 spike protein-specific antibody levels in serum, as measured by ELISA. • GMTs of serum SARS-CoV-2spike protein antibodies, as measured by ELISA, in subjects who did not get exposed to SARS-CoV-2 before the trial, or during the trial before the applicable sample was collected, as measured by ELISA to SARS-CoV-2 N-antigen. • Seroconversion for SARS-CoV-2 neutralizing antibodies, as measured by an activity assay. • Individual SARS-CoV-2 neutralizing antibody levels in serum. • GMTs of serum SARS-CoV-2 neutralizing antibodies, as measured by an activity assay, in	Immunogenicity Set

Objectives	Endpoints	Population
	subjects who did not get exposed to SARS-CoV-2 before the trial, or during the trial before the applicable sample was collected, as measured by ELISA to SARS-CoV-2 N-antigen.	
Exploratory		
Cell-mediated immune response • To evaluate the cell-mediated immune response after 1 and 2 dose administrations of CVnCoV at different dose levels in all subjects from the assigned site(s).	• The frequency and functionality of nCoV spike-specific T-cell response after stimulation, as measured by intracellular cytokine staining (ICS) or ELISPOT • Detectable increase in nCoV spike-specific T-cell response.	Immunogenicity Set
Innate immune response • To evaluate the innate immune response after 1 and 2 dose administrations of CVnCoV at different dose levels in all open-label sentinel subjects	• Serum cytokine concentrations, including but not limited to IFN-α, IFN-γ, IL-6, CCL2, IP-10 for open sentinel subjects • Gene expression profiling.	Immunogenicity Set
Evaluation of infection • To identify and assess cases of COVID-19 disease. • To describe the rate of asymptomatic infections with SARS-CoV-2.	• Virologically-confirmed SARS-CoV-2 infection as measured by RT-PCR at clinically defined timepoints throughout the trial. • Asymptomatic SARS-CoV-2 infection as measured by retrospective serology at predefined time points.	Safety Set Immunogenicity Set

* DDS-1 and DDS-2 sets are primarily for the dose escalation phase of the study.

9.5 Population Sets

9.5.1 Safety Set

The safety set will consist of all subjects who received at least one dose of trial vaccine and for whom any post-vaccination safety data are available.

9.5.2 Dose-determining Set 1 (DDS-1)

The DDS-1 will consist subjects, who have either experienced Grade 3 adverse reactions or SAEs considered as related to the trial vaccine according to the Investigator at any time during the first 24 hours, or completed the 24-hour observation period without experiencing Grade 3 adverse reactions or SAEs considered as related to the trial vaccine according to the Investigator. Of particular interest are the 4 first subjects at each dose level. The DDS-1 will be used for the first dose expansion decision at a particular dose level. The minimum vaccination and safety evaluation requirements will have been met if the subject

has received the planned dose of CVnCoV, has been observed for at least 24 hours following the first vaccine administration and has completed the required safety evaluation. Subjects who do not meet these requirements will be regarded as ineligible for inclusion in the dose-determining set. Since DDS-1 is only used during dose escalation, DDS-1 is not reported in the tables but Grade 3 adverse reactions and SAEs considered as related to the trial vaccine according to the Investigator used for the analyses are marked in the listings.

9.5.3 Dose-determining Set 2 (DDS-2)

The DDS-2 will consist of all subjects in the safety set, who have either experienced Grade 3 adverse reactions or SAEs considered as related to the trial vaccine according to the Investigator at any time during the first 60 hours, or completed the 60-hour observation period without experiencing Grade 3 adverse reactions or SAEs considered as related to the trial vaccine according to the Investigator. The DDS-2 set will be used for dose escalation decisions and for decisions to continue enrollment after the 60-hour safety reviews.

The DDS-1 and DDS-2 population are used in the dose escalation phase and not for analysis associated with the statistical analysis plan.

9.5.4 Immunogenicity Set

The immunogenicity set will include all subjects who received at least one dose of trial vaccine and for whom the baseline blood sample and at least 1 post-baseline blood sample are available for analysis.

10.0 Conventions and Derivations

All data obtained in this trial and documented in the CRF will be listed and summarized with sample statistics or frequency tables as appropriate. In all tables, listings and figures (TFLs), the dose groups will be reported from the lowest to the highest dose. The safety and immunogenicity analyses will be done overall and by baseline serology status for SARS-CoV-2.

The statistical analyses will be reported using tables, listings, and figures (TLFs). The International Council for Harmonisation (ICH) numbering convention will be used for all TLFs.

10.1 Baseline and Change from Baseline

Unless otherwise noted, baseline is defined as the last valid measurement at Visit 1: Day 1 prior administration of the trial vaccine.

Change from Baseline is defined as:

Observed result at nominal time point – observed result at baseline.

10.2 Missing Data

Analysis of vaccination data will be done on a valid case basis, i.e., for missing observations, no imputation for missing data, such as last observation carried forward, will be applied. For nCoV spike protein antibodies and Virus Neutralization, concentration values marked as below the cut point (CP) will be set to 0.5*CP.

For the cytokine concentrations the following rules will be used:

- If Value < LLOQ set to 0.5*LLOQ
- If Value "BLQ" set to 0.5*LLOQ
- If Value “0” set to 0.5*LLOQ
- If Value "ALQ" set to ULOQ

No imputation of missing values will be done for any analysis (except the imputation for missing partial dates of AEs and concomitant medication). Reasons for discontinuation from the trial or trial vaccination will be listed and summarized.

Currently no replacement of drop-out subjects is foreseen.

For unsolicited AEs, solicited AEs occurring after Day 8 and concomitant medications, some missing or partially missing variables will be imputed as follows:

For AE or concomitant medication start date:

- If start date year value is missing, the date uncertainty is too high to impute a rational date. Therefore, if year value is missing, the imputed AE start date is set to missing.
- If start date year value is before the vaccination start date year value, started before the vaccination. Therefore:
  - If month is missing, the imputed start date is set to the mid-year point (i.e., 01JULYYYY).
  - If month is not missing, the imputed start date is set to the mid-month point (i.e., 15MONYYYY).
- If start date year value is equal to the vaccination start date year value, the start date month needs to be compared against the vaccination start date month, to determine the imputation rule to apply. Therefore:
  - If month is missing, the imputed month and imputed day is the same as start of vaccination
  - If month is lower than vaccination start date month and start date day is missing, the imputed start date is set to the mid-month point (i.e. 15MONYYYY).
  - If month is equal to the vaccination start date and start date day is missing, the start day will be set to the start day of vaccination.
  - If month is greater than the vaccination start date month and start date day is missing, the imputed start date is set to the beginning of the month (i.e., 01MONYYYY).
- If start date year value is greater than the vaccination start date year value, started after vaccination. Therefore,
  - If month is missing, the imputed start date is set to the year start point (i.e., 01JANYYYY).
  - If start date month is not missing and start date day is missing, the imputed start date is set to the beginning of the month (i.e., 01MONYYYY).
- If after imputation of start and resolution date (see below) a start date is after the resolution date (for example if a missing day of a start date is set to 15 and the resolution date is before the 15th of the same month and year) then the start date will be set to the resolution date

For resolution date:

- If date of resolution is completely missing, it is assumed that it resolved at the date of the end of the trial.
- If year is present, it is assumed that it resolved on 31 December of that year (i.e., 31DECYYYY), or at the end of the trial if earlier.
- If year and month are present, it is assumed that it resolved on the last day of that month, or at the end of the trial if earlier.

No other safety variables will be imputed. In case the number of missing/partial dates for solicited local AEs, solicited systemic AEs or individual solicited AEs is higher than expected for the analysis of durations (in days), a sensitivity analysis will be conducted to assess the impact on the primary endpoint.

10.3 Prior and Concomitant Medications

Prior medications are medications with a start date prior first date of vaccination. Concomitant medications are those ongoing at or starting on or after the start of vaccination.

10.4 Adverse Events (AEs)

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this vaccination.

10.4.1 Solicited AEs

Solicited local AEs (injection site pain, redness, swelling and itching) and solicited systemic AEs (chills, fever, nausea/vomiting, diarrhea, headache, fatigue, myalgia and arthralgia) will be collected on the day of vaccination and the following 7 days for dose 1 and 2 on a specific diary and after that together with all AEs occurring throughout the trial

Solicited AEs will be assessed on an intensity scale of absent, mild, moderate and severe (Table 5 and Table 6).

By definition, all local solicited AEs are considered related to trial vaccination. For solicited systemic AEs, the Investigator will assess the relationship between trial vaccine and each occurrence of each AE.

Table 5 Intensity Grading* for Solicited Local Adverse Events

AE	Grade	Definition
Pain at injection site	0	Absent
	1	Does not interfere with activity
	2	Interferes with activity and/or repeated use of non-narcotic pain reliever >24 hours
	3	Prevents daily activity and/or repeated use of narcotic pain reliever
Redness	0	<2.5 cm
	1	2.5 – 5 cm
	2	5.1 – 10 cm
	3	>10 cm
Swelling	0	<2.5 cm
	1	2.5 – 5 cm and does not interfere with activity
	2	5.1 – 10 cm or interferes with activity
	3	>10 cm or prevents daily activity
Itching	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity
	3	Significant, prevents normal activity
*FDA toxicity grading scale [1].		

Table 6 Intensity Grading* for Solicited Systemic Adverse Events

Adverse Event	Grade	Definition
Fever	0	<38°C
	1	≥38 – 38.4°C
	2	≥38.5 – 38.9°C
	3	≥39°C
Headache	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity and/or repeated use of non-narcotic pain reliever >24 hours
	3	Significant; any use of narcotic pain reliever and/or prevents daily activity
Fatigue	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity
	3	Significant, prevents normal activity
Chills	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity
	3	Significant, prevents normal activity
Myalgia	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity
	3	Significant, prevents normal activity
Arthralgia	0	Absent
	1	Mild, no interference with normal activity
	2	Moderate, some interference with normal activity
	3	Significant, prevents normal activity
Nausea/ Vomiting	0	Absent
	1	Mild, no interference with activity and/or 1 – 2 episodes/ 24 hours
	2	Moderate, some interference with activity and/or >2 episodes/ 24 hours
	3	Significant, prevents daily activity, requires outpatient IV hydration
Diarrhea	0	Absent
	1	2 – 3 loose stools or <400 g/24 hours
	2	4 – 5 stools or 400 – 800 g/24 hours
	3	6 or more watery stools or >800 g/24 hours or requires outpatient IV hydration

*FDA toxicity grading scale [1]; IV = Intravenous.

10.4.2 Unsolicited Adverse Events

Unsolicited AEs occurring on the day of vaccination and the following 28 days will be collected. For unsolicited AEs occurring on the day of dose 2 the following rule will be used:

If the AE is occurring on the day of dose 2 but before vaccination the AE will be presented with AEs for dose 1, if the AE is occurring on day of dose 2 but on or after vaccination it will be presented with AEs for dose 2. If this rule cannot be applied but the AE occurred on the day of vaccination for dose it will be presented with the AEs for dose 2.

10.4.3 Serious Adverse Events

SAEs are defined as any untoward medical occurrence that results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent disability/incapacity, is a congenital anomaly/birth defect in the offspring of the subject, or is an important medical event.

SAEs and AEs leading to vaccine withdrawal or trial discontinuation will be collected throughout the trial. Non-serious inter-current medical conditions that may affect the immune response to vaccination will also be collected throughout the trial.

10.4.4 Adverse Events of Special Interest

The following events will be considered as AESI during this trial:

- AEs with a suspected immune-medicated etiology (pIMDs, see Appendix 1),
- COVID-19 disease,
- other AEs relevant to SARS-CoV-2 vaccine development or the target disease (see Appendix 2).

AESIs will be collected on CRF throughout the trial

10.4.5 Medically Attended Adverse Events

Medically attended AEs will be collected on the CRF throughout the trial.

10.4.6 Duration Solicited Adverse Events

Duration of solicited local and systemic adverse events are defined as the consecutive days with local/systemic adverse events (days 1 to 8) for the same grade. For example, fatigue with grade 1 occurring on day 2 and 3 and day 7 will be counted as two separate events with the duration 2 days and 1 day. For the duration of solicited local and systemic ongoing beyond Day 8, end of duration is defined as end date on the CRF.

10.4.7 Adverse Reactions

Adverse Reactions are defined as solicited systemic, solicited local and unsolicited adverse events (AEs) considered as related to the trial vaccine according to the investigator.

10.4.8 Adverse Events Related to Standardised and Customized MedDRA Queries

Subjects' AEs are filtered for the following Standardised MedDRA Queries (SMQs):

- Anaphylactic reaction (SMQ)
- Convulsions (SMQ)
- Embolic and thrombotic events (SMQ)
- Hypersensitivity (SMQ)
- Immune-mediated/autoimmune disorders (SMQ)
- Liver related investigations, signs and symptoms (Sub-SMQ)

In addition, two customized MedDRA queries are defined for taste and smelling disorder and events related to paraesthesia, hypoaesthesia, and hyperaesthesia. The related terms are listed in the Appendix 7. The MedDRA SMQs for the specifc terms are not included in the appendix. See official MedDRA documentation.

10.4.9 Credibility Interval and related Estimate

The estimate, using Beta(0.5, 0.5), is calculated as follows:

- (number of reactions + prior a) / (total number of subjects + prior a + prior b).

The lower and upper intervals are calculated as follows for a given number of reactions:

- Using SAS: betainv(0.025, number of reactions + prior a, total number of subjects – number of reactions + prior a) and betainv(0.975, number of reactions + prior a, total number of subjects – number of reactions + prior a). The SAS function betainv returns a quantile from the beta distribution.

10.5 Immunogenicity Assessments

10.5.1 Humoral Immune Response

The humoral immune response elicited by the vaccine will be evaluated by measurement of SARS-CoV-2 spike protein-specific antibodies in serum by ELISA and SARS-CoV-2 neutralizing antibodies in serum by an activity assay.

10.5.2 Retrospective SARS-CoV-2 Baseline Serology Status

The retrospective SARS-CoV-2 status is defined as follows for pre-vaccination samples.

Pre-vaccination collected results (RT-PCR, pre-study SARS-CoV-2 serology or antibody results) and N ELISA and Spike ELISA data will be considered. In general, the below algorithm and cut-off values will be used to determine a subject's status at baseline:

- N ELISA IgG GMT of equal or below 250 is defined as retrospectively SARS-CoV-2 seronegative at baseline
- N ELISA IgG GMT of equal or above 2000 is defined as retrospectively SARS-CoV-2 seropositive at baseline
- If N ELISA IgG GMT is >250 and <2000 and Spike ELISA IgG GMT is equal or below 1300 at baseline this is defined as retrospectively SARS-CoV-2 seronegative at baseline, otherwise as retrospectively SARS-CoV-2 seropositive at baseline

If any of the tests (RT-PCR, pre-study SARS-CoV-2 serology or antibody results) is positive at baseline the subject is regarded as seropositive at baseline independently if the algorithm and cut-off values indicate that subject is seronegative.

The baseline is defined as the samples taken at the screening/clinic visit 1 – day 1.

For subjects in which the retrospectively baseline serology status cannot be determined these will not be included in the counts of seroconverted subjects.

This definition is used throughout the endpoints for the sub-analysis.

10.5.3 Subjects not exposed to SARS-CoV-2 during the trial

In addition to being seronegative at baseline according to the definition in section 10.5.2 a subject will be regarded as seropositive at any given time point post-baseline if

- a RT-PCR is positive,
- or N ELISA IgG GMT is equal or above 2000

If a subject is defined as seropositive at any given visit, the subject will remain seropositive.

10.5.4 Evaluation of Infection

Virologically-confirmed SARS-CoV-2 infections are measured by RT-PCR at clinically determined time points throughout the trial.

Subjects with asymptomatic SARS-CoV-2 infections will be measured by retrospective serology at predefined time points (see section 10.5.2 and 10.5.3). The selection of asymptomatic subjects will be based on the exclusion of subjects with adverse of events of special interest indicating COVID-19 disease.

10.5.5 Geometric Mean

The ELISA and virus naturalizing antibodies are expressed as GMT (geometric mean of reciprocal duplicate dilutions). It is assumed that the data is skewed hence the geometric mean (GM) and geometric standard deviation (GSD) will be presented. The GM is calculated as the anti-logarithm of the mean of the log-transformed data. The GSD will be calculated as the anti-logarithm transformation of the standard deviation of the log-transformed data.

10.5.6 Seroconversion SARS-CoV-2 Spike and SARS-CoV-2 Neutralizing Antibodies

In subjects who did not get exposed to SARS-CoV-2 before the trial or during the trial before the applicable sample was collected, as measured by ELISA to SARS-CoV-2 N-antigen, seroconversion is defined as an increase in titer in antibodies against SARS-CoV-2 spike or SARS-CoV-2 versus baseline, as per the definition in section 10.5.2

In subjects seropositive for SARS-CoV-2 at baseline the definition for seroconversion is a 2-fold increase in titer in antibodies against SARS-CoV-2 spike protein versus baseline.

10.6 Censoring Rules for Subjects Unblinded and/or Treated with Alternate Licensed/Authorized Vaccine

If during the conduct of study CV-NCOV-001 an alternate licensed/authorized vaccine becomes available to some subjects, based on ethical reasons and requests from health authorities these subjects can request to be unblinded from the study treatment to decide whether they would like to receive the alternate licensed/authorized vaccine. The following censoring rules will be applied to these subjects to avoid any study bias.

- Subjects who are unblinded will be censored for the safety endpoints at the first day after unblinding. Any related follow-up data that is collected from censoring time point forward will be included in the listings output.
- Subjects who are unblinded but decide not to receive the alternate licensed/authorized vaccine and to stay in the study will be analyzed for immunogenicity as planned.
- Subjects who are unblinded, but decide to receive the alternate licensed/authorized vaccine, will have their immunogenicity data censored at the first day after receiving the alternate licensed/authorized vaccine. However, any related follow-up data that is collected from this time point forward will be included in the listings output.
- Subjects who received an alternate licensed/authorized vaccine without or before being unblinded or being part of the open-label group will be censored for safety and immunogenicity at the first day after receiving the alternate licensed/authorized vaccine. As described above, data collected from the censoring timepoint forward will be included in the listings output.

This is summarized in Table 7.

Table 7 Censoring Rules for Unblinded Subjects

Analysis	Treatment Received in CV-NCOV-001	Alternate Licensed/Authorized Vaccine Received?	Censoring Rule
Safety	CVnCoV	No	Censored at the first day after unblinding or at the first day after receiving the alternate licensed/authorized vaccine, whichever is earlier
		Yes	
	Placebo	No	
		Yes	
Immunogenicity	CVnCoV	No	Analyzed as planned
		Yes	Censored at the first day after receiving the alternate licensed/authorized vaccine.
	Placebo	No	Analyzed as planned
		Yes	Censored at the first day after receiving the alternate licensed/authorized vaccine

The following details are to be followed regarding censoring the day after unblinding:

- Unblinding day (= Pacific Time Zone) must be adjusted to the subject's time zone.
- Unblinding time does not matter for anything apart from the above time zone normalization. (As case start time is not collected.)
- If the case start day and the unblinding day occur on the same day the case will count for the analysis. (Censoring starts at the day after the unblinding.)

The following details are to be followed regarding censoring the day after receiving the alternate licensed/authorized vaccine:

- Times are not relevant as neither alternate licensed/authorized vaccine time nor case start time are collected.
- If alternate licensed/authorized vaccine date is complete then censoring starts at first day after the alternate licensed/authorized vaccine day.
- If alternate licensed/authorized vaccine date is incomplete (only day missing) then censoring starts after this month.
- If alternate licensed/authorized vaccine date is incomplete (month missing, year available) then censoring starts after this year.
- If alternate licensed/authorized vaccine date is completely missing then no censoring can be made.

As these imputations of incomplete licensed/authorized vaccine dates are not an ideal solution this is only to be considered as a fallback solution in case complete licensed/authorized vaccine dates cannot be retrieved and an interim workaround for unclean data during study conduct.

All incomplete or missing licensed/authorized vaccine dates need to be queried.

11.0 Interim Analyses, DSMB and iSRC

11.1 Interim Analyses

At least one interim analyses may be performed for this trial. The analyses will be based on a data snapshot. The first interim analyses will include 135 known pre-trial seronegative subjects (excluding subjects from DE003 site in which known pre-trial seropositive subjects are enrolled) who were taking part in the trial until at least day 43. The dose levels 2, 4, 6 and 8 µg will be included. The scope for the first interim analysis will not include the entire sub-analysis of subjects retrospectively SARS-CoV-2 seronegative at baseline, and for subjects retrospectively SARS-CoV-2 seropositive at baseline.

As this trial is of exploratory nature and no inferential statistics are planned, no adjustment for multiple testing will be done. Depending on the evolving state of the current pandemic and the public heath need, an early analysis may be performed, and a study report based on interim data may be written to engage with collaborators and/or regulators. Each dose escalation step is considered as an interim analysis (refer to Section 8.1.1 for the dose escalation steps).

11.2 Safety Monitoring Committees

Specified safety data will be reviewed by an iSRC and an external and independent DSMB. The iSRC will consist of the Principal Investigator at each trial site, the Medical Monitor and medical representatives of the Sponsor. Details on the composition, objectives, and role and responsibilities of the iSRC and DSMB will be described in separate charters, agreed with the respective committee members and Sponsor. The charters will also define and document the content of the safety and/or immunogenicity summaries, and general procedures (including communications). The iSRC and DSMB review will be based on unblinded data.

The iSRC and DSMB Chair will review all available safety data obtained during at least 24 hours post-vaccination for each open-label sentinel subject vaccinated on the first vaccination day per dose level, and assess for Grade 3 adverse reaction(s) and any SAE considered as related to the trial vaccine according to the Investigator. Based on this review, the iSRC and DSMB Chair will decide on the enrollment of the subjects planned on the next vaccination day within each dose level. For each dose level, the iSRC will review at least 24-hour post-vaccination safety data from the first 4 seropositive subjects. Vaccination of additional seropositive subjects at that dose level and/or vaccination of subjects with SARS-CoV-2 positive serology at a higher dose level will only proceed upon favorable outcome of this iSRC review. The iSRC will also perform ongoing safety assessments for subjects beyond the first 24 hours on all available data.

In addition, the iSRC together with the DSMB will review all available safety data obtained during at least 60 hours post-vaccination for each subject to recommend on continuation of enrollment at the same dose level and dose escalation. If no stopping rule is met, the DSMB Chair can decide to allow trial progression without obtaining a DSMB quorum.

During the observer-blind placebo-controlled phase at each dose level, if >4 subjects experienced Grade 3 adverse reaction(s) after administration of CVnCoV at a particular dose level, vaccination will be put on hold for all subjects in the applicable dose level and any higher dose level. The DSMB will perform a

comprehensive review of all relevant safety and reactogenicity data before making a decision to stop, continue or modify vaccination (including dose-de-escalation) with this and any higher dose level.

If any SAE considered as related to the trial vaccine according to the Investigator occurs at any moment during the trial, all vaccinations will be put on hold. The DSMB will perform a comprehensive review of all relevant safety and reactogenicity data before making a decision to stop, continue or modify vaccination (including dose-de-escalation).

If a stopping rule or trial suspension rule is limited to subjects with SARS-CoV-2 positive serology, vaccination will only be put on hold for subjects with SARS-CoV-2 positive serology.

The DSMB may recommend additional measures including modification or halt of the trial. After completion of vaccination for all subjects, the DSMB will continue to review safety data every 3 months, or more frequently if needed based on the safety data.

12.0 Statistical Methods

Due to the exploratory nature of this trial, only descriptive statistics will be used. No confirmatory statistical inference will be performed.

All data collected during this study will be displayed in data listings, unless otherwise specified. Data listings will be sorted by dose level and subject identifier. Screening failures will be excluded from all listings and tables if not otherwise stated. Listings will include all relevant assigned/derived variables.

Unless otherwise noted, categorical variables will be summarized using counts and percentages. Descriptive statistics (mean, median, standard deviation (SD), Q1, and Q3, minimum and maximum values) for continuous variable will be presented. Mean, median, Q1 and Q3 will be presented to 1 decimal more than original data. SD will be presented with 2 decimals more than original data. Minimum and maximum will match the decimal points in the original data.

Descriptive summaries and tabulations will be presented overall and by age groups (age 18-40, age 41-60) unless otherwise specified.

All data summaries and tabulations will be prepared using SAS Version 9.4 or higher.

12.1 Subject Disposition

The number and percentage of subjects screened, randomized and treated with the vaccine in the study will be presented with the number and percentage of subjects who withdrew from the study prematurely and a breakdown of the corresponding reasons for early termination and discontinuation.

The number and percentage of subjects included in each analysis set will be provided. Reasons for exclusion from each analysis set will not be tabulated, but will be listed. Subjects not fulfilling any inclusion/exclusion criteria will be listed only.

Subject visits will be listed only.

12.2 Important Protocol Deviations

Per PRA processes, protocol deviations data will be entered into PRA system of record (PSO). The trial team and the Sponsor will conduct on-going reviews of the deviation data from PSO and the resulting set of evaluable subjects throughout the trial, adjusting the deviation criteria as seems appropriate.

Protocol deviation data will be reviewed prior to each formal analysis (i.e. interim analyses of final analyses) and if applicable important deviations leading to elimination of subjects from analysis sets will be identified. If applicable detailed definitions and further guidance on additional programmatic approaches for identification of protocol deviations and/or other criteria will be provided in an appendix to the final SAP.

The detailed definitions of important protocol deviations leading to elimination of subjects from analysis sets will be provided in the final version of the SAP and/or in the final signed minutes of the data review meetings prior to each formal analysis and prior to database lock.

Protocol deviation data will be listed only.

12.3 Demographic and Baseline Characteristics

Demographic characteristics to be summarized will include gender, ethnicity, race, age at screening (years), age group (18 – 40 years, 41-60 years), baseline immune status measured retrospectively, height (cm), weight (kg), BMI (kg/m^2) and childbearing potential.

12.4 Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) using version 24.1. Medical history will be listed only.

12.5 Trial Vaccine

12.5.1 Trial Vaccine Exposure

The administrations of CVnCoV or placebo will be listed and the number of vaccinations will be summarized.

12.5.2 Prior and Concomitant Medications and Vaccinations

Prior and concomitant medication will be summarized with number and percentage and listed according to WHODRUG (Version September 2021 Global B3 or newer), by Anatomical Therapeutic Chemical (ATC). Prior vaccinations will be listed according to WHODRUG (Version September 2021 Global B3), by Anatomical Therapeutic Chemical (ATC).

12.6 Primary Analysis

The primary safety analysis for the dose escalation phase will be conducted by the sponsor. The primary descriptive safety analysis for the dose escalation will be conducted by PRA. Of primary interest for the dose escalation phase are the following endpoints:

- Frequencies of Grade 3 adverse reactions and any serious adverse event (SAE) considered as related to the trial vaccine within at least 24 hours after the first vaccination by dose level.
- Frequencies of Grade 3 adverse reactions and any SAE considered as related to the trial vaccine within at least 60 hours after the first vaccination by dose level.

12.6.1 Primary Safety Analysis for Dose Escalation Phase

The assumption is that the rate of subjects with Grade 3 adverse reactions will be similar in subjects independent of baseline serology status (seronegative or seropositive) and therefore similar in subjects with no history of COVID-19 disease and subjects with SARS-CoV-2 positive serology. Both groups will be analyzed together for the primary analysis of Grade 3 adverse reactions. Additional subgroup analyses will be performed for subjects retrospectively SARS-CoV-2 seronegative at baseline and subjects retrospectively SARS-CoV-2 seropositive at baseline.

An adaptive BLRM guided by the EWOC principle will be used for the dose escalation while decisions of dose level expansion in the sentinel groups of 4 subjects is based on a maximum number of 2 subjects with Grade 3 adverse reactions. Of note, dose escalation can also not proceed if any SAE considered as related to the trial vaccine according to the Investigator occurs. The use of Bayesian response adaptive models for Phase 1 studies has been advocated by the EMA adopted guideline on small populations (EMA, 2006) and by Rogatko *et al.*, 2007 [2] and is one of the key elements of the FDA's Critical Path Initiative.

A modified 2-parameter BLRM will be used for dose escalation [3]. All information currently available about the dose-adverse reaction relationship of CVnCoV is summarized in a prior distribution. This prior distribution is then updated after each group of subjects with all of the adverse reactions data available in the safety set from the current trial. Once updated, the distribution summarizes the probability that the true rate of adverse reactions for each dose lies in the following categories:

- 0% to <33%: targeted tolerance; and
- ≥33% to 100%: intolerance.

The EWOC principle mandates that any dose of CVnCoV that has more than a 50% chance of being in the intolerance category is not considered for the next dose administrations [3,4]. A clinical synthesis of the available safety and reactogenicity information (including adverse reactions that are not Grade 3 adverse reactions), laboratory values and, if available, immune response information as well as the recommendations from the Bayesian model and the iSRC/DSMB will be used to determine the dose level(s) for the next group at a dose-escalation teleconference. In any case where there is a change in dose level to be administered to the next enrolled subjects, a new model will be defined using a Meta-Analytic-Predictive prior, based on the observed data.

The frequency of Grade 3 adverse reactions and SAEs considered as related to the trial vaccine according to the Investigator will be tabulated by dose after 24 hours and 60 hours post-vaccination and information about the Grade 3 adverse reactions and SAEs considered as related to the trial vaccine according to the Investigator will be listed by dose and dose level.

12.6.1.1 Bayesian Logistic Regression Model

The objective of the design is to determine the highest dose with less than 50% risk of a true Grade 3 adverse reaction rate being equal to or above 33%. The dose escalation will be guided by a modified Bayesian 2-parameter logistic regression model with overdose control.
The model is formulated as follows:

$$\text{logit}(p(d)) = \log(\alpha) + \beta * \log(d/d^*),$$

where logit(p) = log(p/(1-p)). p(d) represents the probability of having a Grade 3 adverse reaction in the first 24 hours at dose d, d* = 8µg is the reference dose, allowing for the interpretation of α as the odds of an adverse reaction at dose d*, and θ = (log(α), log(β)) with α, β >0 is the parameter vector of the model.
Since a Bayesian approach is applied, a prior distribution π(θ) for the unknown parameter vector θ needs to be specified. This prior distribution will be specified as a multivariate normal distribution, i.e.,

$$\pi(\theta) = MVN(\mu, \Sigma)$$

the multivariate normal distribution with mean vector µ and covariance matrix Σ, with

$$\Sigma_i = \begin{pmatrix} \sigma_{i,11}^2 & \sigma_{i,11}\sigma_{i,22}\rho_i \\ \sigma_{i,11}\sigma_{i,22}\rho_i & \sigma_{i,22}^2 \end{pmatrix}$$

Prior derivation

Since no prior information is available, the uncertainty about the dose-tolerance relationship is expressed by a mixture distribution composed of a main component (*a priori* assuming 10% and 20% adverse reaction probabilities at 2µg and 8µg, respectively), a low tox (*a priori* assuming 5% and 15% adverse reaction probabilities at 2µg and 8µg, respectively) and a high tox component (*a priori* assuming 25% and 50% adverse reaction probabilities at 2µg and 8µg, respectively). All mixture components are attached with high uncertainty expressed by unit variances of the coefficients. A summary of the prior probabilities of Grade 3 adverse reactions at different doses, as well as the corresponding probability of targeted and overdosing, are shown in Table 9. Graphically, the prior medians with accompanying 95% credible intervals are shown in Figure 2. The uncertainty around the medians is large, showing the low amount of in-men information this prior provides.

Table 8 Summary of prior distribution

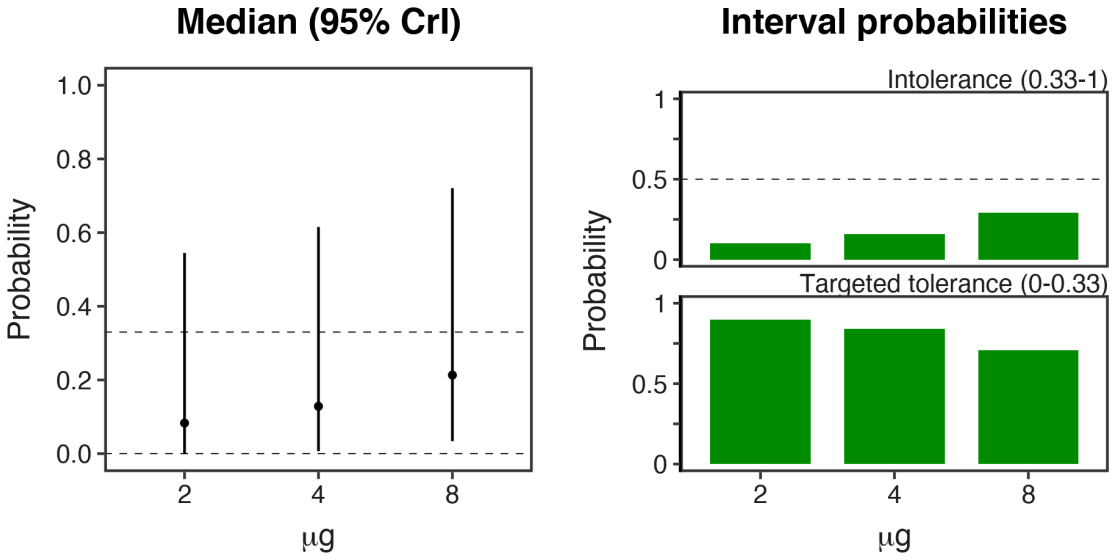
Prior Component	Mixture Weight	Mean Vector	STD Vector	Correlation
Main	0.8	-1.386	1	0
		-0.536	1	
Low tox	0.1	-1.735	1	0
		-0.136	1	
High tox	0.1	0.000	1	0
		-0.233	1	
		-0.761	1	

Table 9 Prior probabilities of Grade 3 adverse reaction at selected doses

Dose (µg)	Probability of true adverse reaction rate in		Mean	STD	Quantiles		
	[0-0.33)	[0.33-1]			2.5%	50%	97.5%
2	0.898	0.102	0.133	0.145	0	0.083	0.545
4	0.841	0.159	0.179	0.161	0.007	0.128	0.615
8	0.708	0.292	0.26	0.185	0.034	0.213	0.721

Doses in bold type meet the overdose criterion, P(overdose) < 0.20.
* STD = standard deviation.

Figure 2 Prior medians and 95% credibility intervals



Cogitars, 26-Mai-2020

The highest acceptable dose may be considered reached if both of the following criteria are fulfilled:

1. The posterior probability of the true Grade 3 adverse reaction rate in the target interval [0%-33%) is at least 50% and the probability of being above the target is below 50%.
2. At least 12 subjects have been vaccinated with this dose level.

Statistical model assessment

The model was assessed using 2 different metrics:

1. **Hypothetical data scenarios:** For various potential data constellations as they could occur in the actual trial, the maximal next doses as allowed by the model and by the 200% escalation limit are investigated. Data scenarios thus provide a way to assess the “on-trial” behavior of the model.
2. **Simulated operating characteristics:** These illustrate for different assumed true dose-tolerance relationships, how often a correct dose would be declared as highest acceptable dose by the model. They are a way to assess the “long-run” behavior of the model.

In summary, the model showed very good behavior as assessed by these metrics. More details can be found in Appendix 3.

12.6.2 Primary Descriptive Safety Analysis

The descriptive safety analyses will be performed for all subjects, for subjects SARS-CoV-2 seronegative at baseline, and for subjects retrospectively SARS-CoV-2 seropositive at baseline, if not otherwise stated.

12.6.2.1 Adverse Events

- Overall summary of AEs (unsolicited AEs, related unsolicited AEs, SAEs, related SAEs, any intercurrent medical conditions affecting immune response, medically attended AEs, AESIs AEs leading to withdrawal from study), including percentage and frequencies.

- Summary of solicited AEs and maximum grade occurring on the day of vaccination and the following 7 days for dose 1 and dose 2.
- Frequencies and percentages of subjects for individual solicited local AEs terms and maximum grade occurring on the day of vaccination and the following 7 days for dose 1 and dose 2.
- Frequencies and percentages of subjects for individual solicited systemic AEs terms and maximum grade occurring on the day of vaccination and the following 7 days for dose 1 and dose 2.
- Frequencies and percentages of subjects for related individual solicited systemic AEs terms and maximum grade occurring on the day of vaccination and the following 7 days for dose 1 and dose 2.
- Day of first onset, daily summary and duration of solicited local AEs, systemic AEs occurring on the day of vaccination and the following 7 days for dose 1 and dose 2.
- The number and percentage of subjects with solicited Grade 3 adverse reaction(s) or SAEs considered as related to the trial vaccine according to the Investigator with the 95% credibility interval based on the Beta (0.5, 0.5) prior distribution for each dose level and dose will be calculated and summarized.
- Occurrence and maximum grading of unsolicited AEs, and related unsolicited AEs occurring on the day of vaccination and the following 28 days for dose 1 and dose 2.
- Occurrence and maximum grading of SAEs, and related SAEs occurring on the day of vaccination and the following 28 days for dose 1 and dose 2.
- Occurrence of unsolicited AEs, SAEs, related SAEs, intercurrent medical conditions affecting immune response, AESIs, medically attended AEs, AEs leading to study withdrawal throughout the trial.
- Occurrence of AEs related to SMQs as defined in Section 10.4.8 (includes solicited AEs). This analysis will be performed for all subjects only.
- Occurrence of AEs related to customized MedDRA queries as defined in Section 10.4.8 (includes solicited AEs). This analysis will be performed for all subjects only.

Individual local and systemic solicited adverse events will be displayed in by subject figures. The duration of grade 3 solicited AEs will be presented in a figure. Solicited and unsolicited summary tables by gender will be presented.

Unsolicited AEs and SAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA 24.1) by System Organ Class (SOC) and Preferred Term (PTT).

12.6.3 Secondary and Exploratory Endpoints

Descriptive statistics for the secondary and exploratory immunogenicity endpoints will be provided by age category. Data will be presented after each vaccine dose.

12.6.3.1 Secondary Endpoints

The humoral immune response will be evaluated on Day 1, Day 8, Day 15, Day 29, Day 36, Day 43, Day 57, Day 120, Day 211 and Day 393. The outputs related to the secondary endpoints will be based on the immunogenicity set (population). In addition, a separate analysis will be conducted based on the following subset:

- Subjects seronegative at baseline and throughout the trial as defined in section 10.5.
- Subjects who received the dose at day 1 and day 29 with one sample 15 days post dose 2.
- And subjects without any protocol deviations impacting immunogenicity measurements.

Individual values and GMT of SARS-CoV-2 spike protein antibody and receptor-binding domain (RBD) levels and of SARS-CoV-2 neutralizing antibodies, percentages of immune cell populations and cellular responders will be summarized for each dose level in all subjects, in those subjects retrospectively SARS-CoV-2 seronegative at baseline, and in those subjects retrospectively SARS-CoV-2 seropositive at baseline. The GMTs are assumed to be skewed hence for applicable outputs the geometric mean and geometric standard deviation with confidence intervals will be presented.

- The proportion and 95%-confidence intervals (exact Clopper-Pearson) of subjects seroconverting with a 2-fold, a 4-fold, and any increase for SARS-CoV-2 spike protein antibodies , as measured by ELISA.
- Geometric mean (GM), geometric SDs (GDS) and 95%-confidence intervals of serum SARS-CoV-2 spike protein antibodies as well as fold change from baseline.
- The proportion and 95%-confidence intervals (exact Clopper-Pearson) of subjects seroconverting with a 2-fold, a 4-fold, and any increase for SARS-CoV-2 neutralizing antibodies, as measured by an activity assay.
- GM, GSDs and 95%-confidence intervals of serum SARS-CoV-2 neutralizing antibodies, as measured by an activity assay as well as fold change from baseline.

GMTs of serum SARS-CoV-2 spike protein antibodies and SARS-CoV-2 neutralizing antibody levels in serum over time will be presented in the following figures overall and by age group:

- Individual line plots of fold change from baseline by dose level.
- Line plot of geometric mean fold change from baseline by dose level.
- Individual line plots of GMTs by dose level.
- Line plot of GMTs with geometric mean and confidence intervals by dose level.
- Boxplots with median, Q1 and Q3, and whiskers representing minimum and maximum.

12.6.3.2 Exploratory Endpoints

Cell-mediated immune response

Cell-mediated assessments will be performed on Day 1, Day 29, Day 36 and Day 211. Testing on Day 211 will only be performed on subjects who are determined as T-cell responders on Day 29 and/or Day 36.

This data will be listed only.

Innate immune response

The innate immune response will only be evaluated in subjects in the open-label sentinel groups. Measurement of serum cytokines, including but not limited to IFN-α, IFN-γ, IL-6, CCL2 and IP-10, will be performed on Day 1, Day 2, Day 8, Day 29, Day 30 and Day 36. In addition, gene expression changes will be determined using the PAXgene® blood RNA system by transcriptome profiling on Day 1, Day 2, Day 8, Day 29, Day 30 and Day 36.

- Serum cytokine concentrations, including but not limited to interferon (IFN)-α, IFN-γ, interleukin (IL)-6, chemokine ligand (CCL) 2 and IFN-γ-induced protein 10 (IP-10) will be presented with descriptive

statistics including 95%-confidence intervals with change from baseline. Two baseline measurements will be used, Day 1 and Day 29.

- Gene expression profiling as changes in gene transcripts is not part of the statistical analysis plan.

Evaluation of infection

- Number of subjects and percentages with virologically-confirmed SARS-CoV-2 infection as measured by reverse transcription polymerase chain reaction (RT-PCR) at clinically determined time points throughout the trial.
- Number of subjects and percentages with asymptomatic SARS-CoV-2 infection as measured by retrospective serology at predefined time points.

12.6.4 Laboratory Data

Blood samples for determination of hematology, clinical biochemistry and coagulation will be analyzed on Day 1, and one and 7 days after each vaccine dose administration (Days, 2, 8, 30 and 36). At all other visits, safety laboratory assessment will only be done in case an abnormal value was observed at the previous visit if not otherwise stated in the safety laboratory summary outputs.

All laboratory data will be summarized in International System (SI) units. The conversion factors from conventional to SI units will be documented in the Local Lab Conventions document for this study.

Safety laboratory values will be classified into low/normal/high based on laboratory normal ranges. Each parameter will be presented by descriptive statistics (n, mean, SD, median, Q1, Q3, minimum and maximum) at each visit including change from baseline. Shift tables based on normal ranges will be presented for selected visits. All laboratory values will be listed. A separate listing for abnormal lab values will be presented.

The mean value with standard deviation for the safety laboratory parameters will be displayed in a line plot by visit.

12.6.4.1 Hematology

Hematology parameters include Erythrocytes, Hematocrit, Hemoglobin, Platelets, Leukocytes, Basophils (Absolute), Basophils/Leukocytes (%), Eosinophils (Absolute), Eosinophils/Leukocytes (%), Lymphocytes (Absolute), Lymphocytes/Leukocytes (%), Monocytes (Absolute), Monocytes/Leukocytes (%), Neutrophils (Absolute), Neutrophils/Leukocytes (%), Reticulocytes (Absolute), Reticulocytes/Erythrocytes (%), Ery. Mean Corpuscular Hemoglobin (MCH), Ery. Mean Corpuscular HBG Concentration (MCHC) and Ery. Mean Corpuscular Volume (MCV).

12.6.4.2 Chemistry

Clinical Chemistry/Biochemistry parameters include Alanine Aminotransferase, Albumin, Alkaline, Phosphatase, Aspartate Aminotransferase, Bilirubin (Total), Direct Bilirubin, Indirect Bilirubin, C-Reactive Protein, Calcium, Creatinine, Gamma Glutamyl Transferase, Lactate Dehydrogenase, Magnesium, Potassium, Total Protein, Sodium, Urea Nitrogen and Urea.

12.6.4.3 Coagulation

Coagulation parameters include Activated Partial Thromboplastin Time, Prothrombin Intl. Normalized Ratio and Prothrombin Time.

12.6.4.4 Serum/Urine Pregnancy Test.

For subjects of childbearing potential, a serum pregnancy test will be performed on the day of enrollment. On Day 1 (pre-vaccination) a urine pregnancy test (hCG) will be performed (only required if the serum pregnancy test was performed more than 3 days before). Pregnancy test results will be listed only.

12.6.5 Vital Signs

Vital signs will be summarized descriptively at each study timepoint they are collected, including screening. Change from baseline values will be summarized for the post-vaccination timepoint. Vital signs parameters to be summarized include systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse rate (bpm) and body temperature (°C).

12.6.6 Physical Examinations, ECGs, and Other Observations Related to Safety

12.6.6.1 Physical Examinations

At specific trial visits a complete physical examination will be performed and the results collected if there are clinically significant results. Physical examination results will be listed only.

12.6.6.2 Electrocardiogram

ECG interpretation and clinically significance will be collected on Day 1 for all subjects. Additionally, ECGs should be performed as clinically indicated. ECG results will be listed only.

13.0 References

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2. Rogatko A, Schoeneck D, Jonas W, et al. Translation of innovative designs into phase I trials. J Clin Oncol. 2007 Nov 1;25(31):4982-6.
3. Neuenschwander B, Branson M, Gsponer T.Critical aspects of the Bayesian approach to phase I cancer trials. Stat Med. 2008 Jun 15;27(13):2420-39.
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14.0 Glossary of Abbreviations

LIST OF ABBREVIATIONS

ALQ	Above limit of quantification
AE	Adverse event
AESI	Adverse event of special interest
ANA	Antinuclear antibody
ARI	Acute respiratory infection
BLRM	Bayesian logistic regression model
BLQ	Below limit quantification
BMI	Body mass index
CCL	Chemokine ligand
CEPI	Coalition for Epidemic Preparedness Innovations
CMI	Cell-mediated immunity
CoV	Coronavirus
CRO	Contract research organization
CTL	Cytotoxic T lymphocyte
CVnCoV	Investigational SARS-CoV-2 mRNA vaccine
DDS	Dose-determining set
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DSMB	Data and safety monitoring board
E	Envelope
ECG	Electrocardiogram
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EWOC	Escalation with overdose control
FDA	US Food and Drug Administration
FIH	First-in-human
GCP	Good Clinical Practice
GMT	Geometric mean titer
hCG	Human chorionic gonadotropin

IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	Intracellular cytokine staining
IEC	Independent Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IM	Intramuscularly
IMP	Investigational medicinal product
IP-10	IFN-γ-induced protein 10
IRB	Institutional Review Board
iSRC	Internal safety review committee
IUD	Intrauterine device
IUS	Intrauterine systems
IV	Intravenous
IVRS	Interactive voice response system
LLOQ	Lower limit of quantification
LNP	Lipid nanoparticles
M	Membrane
MedDRA	Medical Dictionary for Regulatory Activities
MERS	Middle East Respiratory Syndrome
mRNA	Messenger ribonucleic acid
N	Nucleocapsid
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
pIMD	Potential immune-mediated disease
PT	Preferred Term
RBD	Receptor binding domain
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
S	Spike
SAE	Serious adverse event

SAP	Statistical analysis plan
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SMQ	Standardised MedDRA Query
SOC	System Organ Class
SOP	Standard operating procedure
TSH	Thyroid stimulating hormone
VDE	Vaccine dependent disease enhancement
WHO	World Health Organization

15.0 Appendices

Appendix 1 Potential Immune-Mediated Diseases

Current list of pIMDs:

Gastrointestinal disorders:
o Celiac disease
o Crohn's disease
o Ulcerative colitis
o Ulcerative proctitis
Liver disorders:
o Autoimmune cholangitis
o Autoimmune hepatitis
o Primary biliary cirrhosis
o Primary sclerosing cholangitis
Metabolic diseases:
o Addison's disease
o Autoimmune thyroiditis (including Hashimoto thyroiditis)
o Diabetes mellitus type I
o Grave's or Basedow's disease
Musculoskeletal disorders:
o Antisynthetase syndrome
o Dermatomyositis
o Juvenile chronic arthritis (including Still's disease)
o Mixed connective tissue disorder
o Polymyalgia rheumatic
o Polymyositis
o Psoriatic arthropathy
o Relapsing polychondritis
o Rheumatoid arthritis
o Scleroderma, including diffuse systemic form and CREST syndrome
o Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
o Systemic lupus erythematosus
o Systemic sclerosis
Neuro-inflammatory disorders:
o Acute disseminated encephalomyelitis, including site specific variants (e.g., non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis)
o Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy)
o Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
o Immune-mediated peripheral neuropathies, Parsonage–Turner syndrome and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and polyneuropathies associated with monoclonal gammopathy
o Multiple sclerosis

o Narcolepsy
o Optic neuritis
o Transverse Myelitis
Skin disorders:
o Alopecia areata
o Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis
o Cutaneous lupus erythematosus
o Erythema nodosum
o Morphoea
o Lichen planus
o Psoriasis
o Sweet's syndrome
o Vitiligo
Vasculitides:
o Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
o Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis
Others:
o Antiphospholipid syndrome
o Autoimmune hemolytic anemia
o Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
o Autoimmune myocarditis/cardiomyopathy
o Autoimmune thrombocytopenia
o Goodpasture syndrome
o Idiopathic pulmonary fibrosis
o Pernicious anemia
o Raynaud's phenomenon
o Sarcoidosis
o Sjögren's syndrome
o Stevens-Johnson syndrome
o Uveitis

Appendix 2 Adverse Events of Special Interest for SARS-CoV-2 Vaccines

Current list of AESIs (based on Brighton Collaboration via CEPI's Safety Platform for Emergency vACcines [SPEAC] Project):

Immunological disorders:
- o Anaphylaxis
- o Vasculitides
- o Enhanced disease following immunization
- o Multisystem inflammatory syndrome in children

Respiratory disorders:
- o Acute respiratory distress syndrome
- o COVID-19 disease

Cardiac disorders:

Acute cardiac injury including:
- o Microangiopathy
- o Heart failure and cardiogenic shock
- o Stress cardiomyopathy
- o Coronary artery disease
- o Arrhythmia
- o Myocarditis, pericarditis

Hematological disorders:
- o Thrombocytopenia

Coagulation disorder:
- o Deep vein thrombosis
- o Pulmonary embolus
- o Cerebrovascular stroke
- o Limb ischemia
- o Hemorrhagic disease

Renal disorders:
- o Acute kidney injury

Gastrointestinal disorders
- o Liver injury

Neurological disorders:
- o Generalized convulsion
- o Guillain-Barré Syndrome
- o Acute disseminated encephalomyelitis
- o Anosmia, ageusia
- o Meningoencephalitis

Dermatologic disorder:
- o Chilblain-like lesions
- o Single organ cutaneous vasculitis
- o Erythema multiforme

Other:
- o Serious local/systemic AR following immunization

Appendix 3 Statistical Appendix Including Model Performance and Data Scenarios

The model was assessed by 2 different metrics: hypothetical on-trial data scenarios and long-run operating characteristics.

Hypothetical Data Scenarios

Exemplary hypothetical data scenarios are shown in Table 10 to Table 12. These scenarios reflect potential on-trial data constellations and related escalation as allowed by the model and the 200% escalation limit. For each scenario, the probability of overdosing for the current dose, as well as the next potential dose and related probabilities of target-dosing are shown.
These settings illustrate the adaptive behavior of the model which takes into account all observed data from previous dose levels and show a reasonable behavior of the model.

Table 10 **Escalation from 2µg to 4µg:** Escalate to 4µg if not more than 4 in 12 subjects experience a Grade 3 adverse reaction.

Scenario	Dose	#AR	#Pat	CD - P(OD)	Next Dose	ND - P(TD)	ND - P(OD)
1	2	0	12	0.000	4	0.994	0.006
2	2	4	12	0.269	4	0.524	0.476
3	2	5	12	0.496	2	0.504	0.496

CD=Current dose
P(OD)= Probability of overdose
ND= Next dose
P(TD)= Probability of target dose
P(OD)= Probability of overdose

Table 11 **Escalation from 4µg to 8µg:** Only borderline scenarios are provided with maximum number of Grade 3 adverse reactions at 4µg which just allow to escalate. 3 to 4 adverse reactions at 4µg support escalation to 8µg depending on the outcome on previous dose levels.

Scenario	Dose	#AR	#Pat	CD - P(OD)	Next Dose	ND - P(TD)	ND - P(OD)
3	2	0	12				
	4	4	12	0.052	8	0.599	0.401
4	2	1	12				
	4	4	12	0.106	8	0.568	0.432
5	2	2	12				
	4	3	12	0.094	8	0.658	0.342
6	2	3	12				
	4	3	12	0.193	8	0.558	0.442
7	2	4	12				
	4	2	12	0.18	8	0.61	0.39

Table 12 **Confirmation of 8µg:** Scenarios represent the situations with the maximum number of Grade 3 adverse reactions at 8µg which still allow to confirm the highest dose level. 3 to 5 adverse reactions at 8µg support confirmation of safety of 8 µg depending on the outcome in previous dose levels.

Scenario	Dose	#AR	#Pat	CD - P(OD)	Next Dose	ND - P(TD)	ND - P(OD)
8	2	0	12				
	4	0	12				
	8	5	12	0.391	8	0.609	0.391
9	2	0	12				
	4	2	12				
	8	5	12	0.452	8	0.548	0.452
10	2	0	12				
	4	3	12				
	8	4	12	0.328	8	0.672	0.328
11	2	0	12				
	4	4	12				
	8	4	12	0.402	8	0.598	0.402
12	2	1	12				
	4	1	12				
	8	5	12	0.321	8	0.679	0.321
13	2	1	12				
	4	3	12				
	8	5	12	0.497	8	0.503	0.497
14	2	1	12				
	4	4	12				
	8	4	12	0.433	8	0.567	0.433
15	2	2	12				
	4	2	12				
	8	5	12	0.431	8	0.569	0.431
16	2	2	12				
	4	3	12				
	8	4	12	0.375	8	0.625	0.375
17	2	2	12				
	4	4	12				
	8	4	12	0.484	8	0.516	0.484
18	2	3	12				
	4	3	12				
	8	4	12	0.44	8	0.56	0.44

Operating Characteristics

Operating characteristics are a way to assess the long-run behavior of a model. Under an assumed true dose-tolerance curve, metrics such as the probability of recommending a dose with true Grade 3 adverse reaction rate in the target interval can be approximated via simulation. Table 13 describes 3 assumed true scenarios which were used to assess the operating characteristics of the model. These scenarios reflect a wide range of possible cases as follows:

- Scenario 1 (P): aligned with prior means
- Scenario 2 (H): high-intolerance scenario

- Scenario 3 (LH): low-intolerance followed by high-intolerance

Table 13 Assumed True Dose-Tolerance Scenarios

Scenario				
	Dose	2	4	8
1(P)	P(Grade 3 adverse reactions)	**0.13**	**0.18**	**0.26**
2(H)		**0.30**	0.50	0.60
3(LH)		**0.15**	**0.30**	0.45

Bold numbers indicate true adverse reaction rates in the target interval [0-0.33).

For each of these scenarios, 500 trials were simulated. It was then assessed how often a dose was declared safe with true Grade 3 adverse reaction rate in the targeted or over-dose range. Furthermore, the average, minimum and maximum number of subjects per trial and the average number of Grade 3 adverse reactions per trial are reported. Results are shown in Table 14.

Table 14 Hypothetical Data Scenarios

Scenario	% of trials recommending a dose with true Grade 3 adverse reaction rate in		Stopped	# Subjects	# Grade 3 adverse reactions
	Target dose	Overdose	% of trials	Mean (Min - Max)	Mean (Min - Max)
1 (P)	98.2	0	1.8	33.58 (4 - 36)	6.21 (0 - 11)
2 (H)	56.4	21	22.6	18.79 (4 - 36)	7.18 (3 - 15)
3 (LH)	74.2	24	1.8	28.75 (4 - 36)	7.66 (3 - 13)

In Scenario 1, which reflects the case that the true dose-tolerance relationship is aligned with prior means, 98.2% of the simulated trials declared a dose as safe with true Grade 3 adverse reaction rate in the targeted dose range, while 1.8% of the simulated trials were stopped without declaring a dose to be safe.
In Scenario 2 (high-intolerance scenario), the starting dose has already 30% probability of observing at least 1 Grade 3 adverse reaction at the first dose level. This contributes to the increased percentage of 22.6% of all simulated trials for which the trial is stopped since none of the doses is considered tolerable anymore. This is an expected situation for a high-intolerance scenario. Despite the high intolerance probabilities assumed, only 21% of the simulated trials declared a dose safe with true Grade 3 adverse reaction rate in the overdose range.

Scenario 3, 74.2% of the simulated trials declared a dose safe with true Grade 3 adverse reaction rate in the targeted dose range and in only 1.8% the trial was stopped, while in 24% of the trials, a safe dose in the overdose range was declared.
The mean subject numbers ranged from 18.79 subjects (high scenario) to 33.58 subjects (prior means scenario) and the maximum number of subjects was 36. Mean numbers of grade 3 ARs ranged from 6.21 to 7.66.

In summary, the operating characteristics demonstrate a good precision of the highest acceptable dose determination.

Appendix 4 Inclusion/Exclusion Criteria

Inclusion Criteria

Subjects will be enrolled in this trial only if they meet **all** of the following criteria:

1. Healthy male and female subjects aged 18 to 60 years inclusive.

   Healthy subject is defined as an individual who is in good general health, not having any mental or physical disorder requiring regular or frequent medication.
2. Expected to be compliant with protocol procedures and available for clinical follow-up through the last planned visit.
3. Physical examination and laboratory results without clinically significant findings according to the Investigator's assessment.
4. Body mass index (BMI) ≥18.0 and ≤30.0kg/m^2 (≥18.0 and ≤32.0kg/m^2 for subjects with SARS-CoV-2 positive serology).
5. Females: At the time of enrollment, negative human chorionic gonadotropin (hCG) pregnancy test (serum) for women presumed to be of childbearing potential on the day of enrollment. On Day 1 (pre-vaccination): negative urine pregnancy test (hCG), (only required if the serum pregnancy test was performed more than 3 days before).
6. Females of childbearing potential must use highly effective of birth control from 1 month before the first administration of the trial vaccine until 3 months following the last administration. The following methods of birth control are considered highly effective when used consistently and correctly:
   - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal);
   - Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable);
   - Intrauterine devices (IUDs);
   - Intrauterine hormone-releasing systems (IUSs);
   - Bilateral tubal occlusion;
   - Vasectomized partner;
   - Sexual abstinence (periodic abstinence [e.g., calendar, ovulation, symptothermal and post-ovulation methods] and withdrawal are not acceptable).

Exclusion CriteraSubjects will not be enrolled in this trial if they meet **any** of the exclusion criteria.
The following criterion applies to all open-label sentinel subjects:

7. Subjects with confirmed SARS-CoV-2 positive serology as confirmed by testing at enrollment.

<u>The following criteria apply to all subjects, except those with SARS-CoV-2 positive serology:</u>

8. Subjects considered at the Investigator's discretion to be at increased risk to acquire COVID-19 disease (including, but not limited to, health care workers with direct involvement in patient care or care of long-term care recipients).

9. History of confirmed COVID-19 disease or known exposure to an individual with confirmed COVID-19 disease or SARS-CoV-2 infection within the past 2 weeks.

The following criteria apply to all subjects:

10. Use of any investigational or non-registered product (vaccine or drug) other than the trial vaccine within 28 days preceding the administration of the trial vaccine, or planned use during the trial period.

11. Receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment in this trial or planned receipt of any vaccine within 28 days of trial vaccine administration.

12. Receipt of any investigational SARS-CoV-2 or other CoV vaccine prior to the administration of the trial vaccine.

13. Any treatment with immunosuppressants or other immune-modifying drugs (including, but not limited to, corticosteroids, biologicals and Methotrexate) within 6 months prior to the administration of the trial vaccine or planned use during the trial, with the exception of topically-applied steroids. Corticosteroids used in the context of COVID-19 disease of subjects with SARS-CoV-2 positive serology are not exclusionary.

14. Any medically diagnosed or suspected immunosuppressive or immunodeficient condition based on medical history and physical examination, including known human immunodeficiency virus infection, hepatitis B virus infection and hepatitis C virus infection.

15. History of a pIMD.

16. History of angioedema.

17. Any known allergy, including allergy to any component of CVnCoV or aminoglycoside antibiotics. A history of hay fever or seasonal allergies (pollinosis) that does not require current treatment (e.g., anti-histamines) during the vaccination period (1 month before first vaccination until 1 month after last vaccination) is not exclusionary.

18. History of or current alcohol and/or drug abuse.

19. Subjects who are active smokers, were active smokers within the last year (including any vaping in the last year) or have a total smoking history ≥10 pack years.

20. Acute or currently active SARS-CoV-2 infection as confirmed by reactive PCR within 3 days of first trial vaccine administration.

21. History of confirmed SARS or MERS.

22. Administration of immunoglobulins (Igs) and/or any blood products within the 3 months preceding the administration of any dose of the trial vaccine.

23. Presence or evidence of significant acute or chronic medical or psychiatric illness. Significant medical or psychiatric illnesses include but are not limited to:
    - Respiratory disease (e.g., chronic obstructive pulmonary disease, asthma) requiring daily medications currently or any treatment of respiratory disease exacerbations (e.g., asthma exacerbation) in the last 5 years.
    - Respiratory disease with clinically significant dyspnea in the last 5 years (except COVID-19 disease in subjects with SARS-CoV-2 positive serology).

- Asthma medications: inhaled, oral, or intravenous (IV) corticosteroids, leukotriene modifiers, long- and short-acting beta agonists, theophylline, ipratropium, biologics.
- Significant cardiovascular disease (e.g., congestive heart failure, cardiomyopathy, ischemic heart disease, history of stroke, peripheral artery disease, pulmonary embolism) or history of myocarditis or pericarditis as an adult.
- Elevated blood pressure or hypertension, even if well-controlled.
- Diabetes mellitus type 1 or 2.
- History of any neurological disorders or seizures including Guillain-Barré syndrome, with the exception of febrile seizures during childhood.
- Current or past malignancy, unless completely resolved without sequelae for >5 years.

24. Foreseeable non-compliance with protocol as judged by the Investigator.
25. For females: pregnancy or lactation.
26. History of any anaphylactic reactions.
27. Subjects with impaired coagulation or any bleeding disorder in whom an IM injection or a blood draw is contraindicated.
28. Subjects employed by the Sponsor, Investigator or trial site, or relatives of research staff working on this trial.

Appendix 5 List of TFLs

A complete list of all TFLs is provided in a separate document CV-NCOV-001 Statistical Analysis Plan. The document will be managed outside of this SAP.

Appendix 6 Identification of Protocol Deviations Leading to Exclusion from Analysis Sets

Details on the identification of protocol deviations leading to exclusion of subjects from analysis sets are described in a separate document CV-NCOV-001 Statistical Analysis Plan. The document will be maintained outside of this SAP.

Appendix 7 MedDRA Terms for CMQs

Based on MeddRA Version 24.1

A. Customized MedDRA Queries related to taste and smelling disorder

Preferred Term
Ageusia
Anosmia
Dysgeusia
Hypergeusia

Hypogeusia
Hyposmia
Parosmia
Taste disorder

B. Customized MedDRA Querie related to paraesthesia, hypoaesthesia, and hyperaesthesia

Preferred Term
Anal hypoaesthesia
Anal paraesthesia
Dental paraesthesia
Eye paraesthesia
Genital hyperaesthesia
Genital hypoaesthesia
Genital paraesthesia
Hemihyperaesthesia
Hemiparaesthesia
Hyperaesthesia
Hyperaesthesia eye
Hyperaesthesia teeth
Hypoaesthesia
Hypoaesthesia eye
Hypoaesthesia oral
Hypoaesthesia teeth
Intranasal hypoaesthesia
Intranasal paraesthesia
Oral hyperaesthesia
Paraesthesia
Paraesthesia ear
Paraesthesia mucosal
Paraesthesia oral
Pharyngeal hypoaesthesia
Pharyngeal paraesthesia
Thermohyperaesthesia
Thermohypoaesthesia