COVID-*e*Vax

STUDY TITLE

A PHASE I/II STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF COVID-*e*VAX, A CANDIDATE PLASMID DNA VACCINE FOR COVID-19, IN HEALTHY ADULT VOLUNTEERS

CLINICAL TRIAL CODE: COV-1/2-01

EudraCT Number 2020-003734-20

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Document Revision History:
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INVESTIGATOR PROTOCOL AGREEMENT PAGE

I, the undersigned, after having read the Clinical Trial Protocol COV-1/2-01, entitled "A Phase I/II study to assess the safety and immunogenicity of COVID-*e*VAX, a candidate plasmid DNA vaccine for COVID-19, in healthy adult volunteers", Version No. 01 dated 10 November 2020:

- confirm the agreement to conduct the trial in compliance with this Clinical Trial Protocol, with ICH Good Clinical Practice guideline and with all applicable Regulatory Requirements;
- acknowledge that I am responsible for overall study conduct and agree to personally conduct or supervise the described clinical trial;
- agree to ensure that the staff assisting me in the conduct of the study are informed about their obligations;
- agree to ensure direct access to source documents to study monitors, auditors and Regulatory Authorities;
- confirm herewith that Rottapharm Biotech S.r.l. is allowed to enter and utilize my professional contact details and position in an electronic database for internal purposes and for submission to Health Authorities worldwide;
- agree that all the information communicated to me by Rottapharm Biotech S.r.l. and the data generated in this study are the exclusive property of Takis S.r.l. and Rottapharm Biotech S.r.l., and ensure that the same shall be kept strictly confidential by me or any other person involved in the study and shall not be disclosed by me or such person to any third party without the prior written authorization of Takis S.r.l. and Rottapharm Biotech S.r.l.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYNOPSIS

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Name of Product: COVID-*e*Vax

Study Title: A Phase I/II study to determine safety and immunogenicity of COVID-*e*Vax, an anti-SARS-CoV-2 plasmid DNA vaccine

Protocol Code Number: COV-1/2-01

EudraCT Number: 2020-003734-20

Study Centres and Principal Investigators:

Three sites selected in Italy:

- Istituto Nazionale Tumori, IRCCS, Fondazione G. Pascale, Napoli
  Principal Investigator: Paolo Antonio Ascierto
- University of Milano-Bicocca and San Gerardo Hospital, Monza
  Principal Investigator: Paolo Bonfanti
- INMI Lazzaro Spallanzani, Roma
  Principal Investigator: Simone Lanini

Coordinating Investigator:

Coordinating Investigator: Paolo Antonio Ascierto

Co-coordinating Investigators: Paolo Bonfanti and Simone Lanini

Phase of Development: I/II

Introduction:

The study is aimed at assessing the safety and immunogenicity of COVID-*e*Vax, a DNA plasmid-based vaccine whose target antigen is a portion of the S protein of SARS-CoV-2 virus (the Receptor Binding Domain located in the CTD1 of the S1 region of the S protein). In animal models COVID-*e*Vax was safe and induced high immunological humoral and cellular response.

Objectives:

Phase I (Dose Escalation):

Primary Objective:

- To assess the safety and reactogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To identify the dose(s)/schedule(s) to be used in the Phase II (Dose Expansion)

Secondary Objective:

- To preliminarily assess the immunogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

Phase II (Dose Expansion):

Primary Objective:

- To assess the immunogenicity of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

Secondary Objective:

- To assess the duration of the immune response of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To assess the (long-term post-administration) safety of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

Study Design: Multicentre, open-label Phase I/II study with a first-in-human dose escalation part (Phase I study) followed by a dose expansion part (Phase II study)

Experimental Procedure(s):

Phase I Dose Escalation

COVID-*e*Vax will be administered at 3 escalating doses (20 subjects/cohort), in a prime-boost setting, as follows:

- Cohort 1: 0.5 mg prime and 0.5 mg boost (PB), 4 weeks apart - Total dose: 1 mg
- Cohort 2: 1 mg prime and 1 mg boost (PB), 4 weeks apart - Total dose: 2 mg
- Cohort 3: 2 mg prime and 2 mg boost (PB), 4 weeks apart - Total dose: 4 mg

In addition a cohort in a prime setting will also be tested:

- Cohort 4: 2 mg prime (P) - Total dose: 2 mg

In the Phase I part of the study a cohort progression scheme will be used, to ensure the safety of each vaccine dose schedule, by sentinel dosing/dose staggering techniques.

Dosing will begin with 6 sentinel subjects in the Cohort 1. The subjects will be treated according a 1-2-3-subjects scheme, with a 3-day interval, and only in case of no safety concerns after AE and safety laboratory parameters review.
The same applies to the remaining 14 participants in cohort 1, who will be enrolled, in case of no safety concerns in the first 6 subjects, 3 days after enrolment of the 6th sentinel participant.

The next cohort can be opened after AEs and clinical laboratory data review of all participants in the previous cohort for the 7-day period, ideally 8 days after the last subject in the previous cohort was treated.

The same sentinel dosing/cohort completion scheme will be followed also for Cohorts 2 and 3; Cohort 4 can be started after completion of sentinel subjects in Cohort 3.

The best dose schedule in terms of safety and immunogenicity will be selected for expansion. Two dose schedules may be selected, in case of a minimum difference between the best two.

Phase II Dose Expansion

This phase will evaluate the best dose schedule(s) selected in Phase I. Phase II will start after reviewing the safety and immunogenicity data obtained at least 4 weeks after the last vaccination in all subjects enrolled in Phase I.

In both study phases, participants will undergo 2 vaccination sessions (4 weeks apart) or only 1, depending on the assigned dosing schedule.

The Phase I - Dose escalation part of the study encompasses a total of 12 (11 for subjects belonging to P group) clinic visits:

Screening Visit (V1)
Baseline Visit (V2 - D1, 1st vaccination administration)
Day 3 (V3)
Week 1 (V4)
Week 2 (V5)
Week 4 (V6); D29 - 2nd vaccination administration, not applicable for P subjects
Week 5 (V7), 1 week after boost dose, visit not applicable for P subjects
Week 6 (V8)
Week 8 (V9)
Week 12 (V10)
Week 16 (V11)
Week 24 (V12, Final Visit)

The Phase II - Dose expansion part of the study encompasses a total of 10 (9 for subjects belonging to P group) clinic visits:

Screening Visit (V1)
Baseline Visit (V2 - D1, 1st vaccination administration)
Week 1 (V3)
Week 2 (V4)
Week 4 (V5) - D29 - 2nd vaccination administration, not applicable for P subjects
Week 5 (V6), 1 week after boost dose, visit not applicable for P subjects
Week 6 (V7)
Week 8 (V8)
Week 12 (V9)
Week 24 (V10, Final Visit)

Number of Subjects:

A suitable number of subjects will be screened, in order to treat 160 or 240 subjects in the two study Phases.

Phase I: 80 subjects, i.e. 20 in each of the 4 cohorts.

Phase II: Additional subjects will be selected for enrolment in the expansion cohort(s) to reach a sample size of 100 subjects in each expanded dose schedule cohort. Therefore, if only one dose schedule is selected for expansion, a total of additional 80 subjects will be enrolled, while if two dose schedules are selected for expansion, a total of additional 160 subjects will be enrolled.

Study population:

Healthy male and non-pregnant female non-obese subjects, aged 18-65 years, with normal vital signs, ECG and laboratory parameters and with no signs or symptoms of respiratory infection. SARS-COV-2 infection will be excluded before vaccination.

A Male:Female 1:1 ratio (approximately) will be maintained within each cohort.

Study Drug(s), Dose(s), and Mode(s) of Administration:

COVID-*e*Vax will be supplied in sterile vials containing 4mg/mL.

The lower doses, i.e. 0.5 mg and 1 mg, will be obtained by dilution with sterile saline, maintaining an administration volume of 0.5 mL.

The 2 mg dose will be obtained by injection of 0.5 mL of the vial content (no dilution).

COVID-*e*Vax will be administered by intramuscular injections into the deltoid muscle, followed immediately by electroporation (IGEA Cliniporator® and EPSGun, electrical conditions consisting of 4 pulses at voltage amplitude of 40V (corresponding to an electric field strength of 100V/cm), lasting 5 msec, at an interval of 5 msec) at each vaccination visit, once or twice (4 weeks apart), depending on the assigned treatment group.

Study Endpoints:

Phase I

Primary Endpoints

- Incidence of solicited local AEs at the injection site and solicited systemic AEs, through 7 days post-each vaccination.
- Incidence of unsolicited AEs and changes in safety laboratory parameters, through 4 weeks post-last vaccination.

Secondary Endpoints

- through 4 weeks post-last vaccination
  - Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen and SARS-CoV-2 neutralizing antibody titer (Geometric Mean Titer (GMT) and Geometric Mean Fold Rise (GMFR) from baseline).
  - Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2.
  - Percentage of subjects who seroconverted.

- through study completion (6 months)
  - Duration of the immune response on all criteria and parameters

- Incidence of unsolicited AEs

Phase II

Primary Endpoints

See secondary immunogenicity endpoints evaluated through 4 weeks post-last vaccination described for Phase I.

Secondary Endpoints

See primary endpoints described for Phase I.

Duration of the immune response on all criteria and parameters, through study completion (6 months).

Incidence of unsolicited AEs through study completion (6 months).

In both study Phases, all primary and secondary endpoints will also be assessed in the time frame through study completion (6 months).

Statistical Methods:

Sample size

No formal sample size calculation has been performed.

In Phase I, 20 subjects per group are considered sufficient to assess safety and reactogenicity, while preliminarily assessing immunogenicity. This sample size will give a 88% chance of observing at least 1 AE, in case of AEs with 10% underlying incidence, and a 90% chance in case the underlying incidence is 20%. If no AE is observed among 20 subjects, this will provide an 80% confidence that the underlying incidence rate is < 7.7% or a 90% confidence that the underlying rate is < 10.8%.

In Phase II, 80 subjects will be included in each expanded dose schedule cohort. This number is considered sufficient to characterize the safety and immunogenicity of each expanded dose schedule cohort, that would be evaluated in a total of 100 subjects each (considering both study phases). Under seroconversion rates of 70% and 90%, the width of the estimated 95% Confidence Interval (based on a Binomial "exact" calculation) will be 19% and 13%, respectively.

Statistical Analyses

Safety: the analysis of reactogenicity will be based on the incidence of solicited local AEs at the injection site and solicited systemic AEs, summarized by severity for each day post vaccination (Days 1-7) and as the maximum severity over all 7 days. Unsolicited AEs will be summarized by cohort, for the first 28 days after vaccination and then for the overall study period, by MedDRA System Organ Class and Preferred Term.

Immunogenicity: quantitative humoral responses will be expressed as antibody titers, analysed as GMT and GMFR from baseline. Seroconversion rates of both binding and neutralizing antibodies, GMFR and GMT, will be calculated at Day 1 (GMT only) and at each post-baseline timepoint by cohort, and will be summarized graphically. The vaccine induced T-cell response, as assessed by ELISpot, will be summarized at each timepoint by cohort, and will be displayed graphically.

Study duration and Key Decision Points:

The study is planned to start in December 2020.

Key decision points

- Availability of safety and immunogenicity data (4-week) for Phase I and **decision to move to Phase II**: March 2021
- Availability of safety and immunogenicity data (4-week) for Phase II and **decision to move to Phase III**: June 2021

Study completion will occur when all subjects have completed the 6-month observation period (November 2021).

SCHEDULE OF EVALUATIONS: FLOWCHART

Phase I - Schedule of activities and evaluations

Procedure	Screen. -30/-1	Day 1	Day 3	Wk1 (D8 ±1)	Wk2 (D15 ±1)	Wk4 (D29 ±1)	#Wk5 (D8±1 post boost)	Wk6 (D43 ±2)	Wk8 (D57 ±2)	Wk12 (D85 ±3)	Wk16 (D113 ±3)	Wk24 (D169 ±5)
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Informed Consent	X											
Medical history	X	X										
Demographics	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[1]	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X[2]	X	X	X	X[2]	X	X	X	X	X	X
12-lead ECG	X	X[3]				X[3]						
Hematology	X	X	X	X		X	X		X	X		X
Chemistry[4]	X	X	X	X		X	X		X	X		X
Urinalysis	X	X	X	X		X	X		X	X		X
Serology[5]	X											
Nasopharingeal swab for SARS-CoV-2		X[6]				X[7]						
SARS-COV-2 serology (quantitative)	X											
Pregnancy test[8]	X	X[9]				X[9]			X	X		X
Binding antibodies[10]		X		X	X	X	X	X	X	X	X	X
Neutralizing antibodies[10]		X				X			X			X
Cellular immune response[10]		X			X	X			X	X		X
Vaccination		X[11]				X[12]						
Adverse Events	←----------											----------→
Participant Diary		X[13]	X[14]	X[15]		X[13]	X[15]					
Telephone contact		X[16]				X[16]						

#Visit not applicable to subjects belonging to prime group

[1] Full physical examination at screening and last study visit only; targeted examination at other visits, as determined by Investigator or per participant complaints

[2] Vital signs to be performed pre- and 30-min-post- vaccination in subjects undergoing vaccine administrations, at any time for the others

[3] One (1) hour after vaccine administration

[4] Sodium (Na), potassium (K), glucose, BUN or urea, Cr, total bilirubin, ALT, AST, CPK, total proteins, CRP

[5] HIV antibodies, HBsAg, HCV antibodies

[6] Nasopharingeal swab to be performed in the 72 hours before vaccination on D1

[7] Nasopharingeal swab to be performed in the 72 hours before vaccination on D29 for PB groups only

[8] Serum/Urine pregnancy test

[9] On D1 before vaccine administration for all subjects; on D29 before vaccine administration for PB groups, at any time for the others

[10] Details on volumes required for the immunogenicity assessments are provided in section 10

[11] Vaccine administration for all participants

[12] Vaccine administration for participants belonging to PB groups only

[13] Diary hand-over and training on use to participants
[14] Diary check
[15] Diary collection
[16] In the evening of the day of vaccination and 24 hours after vaccination

Phase II - Schedule of activities and evaluations

Procedure	Screen. -30/-1	Day 1	Wk1 (D8 ±1)	Wk2 (D15 ±1)	Wk4 (D29 ±1)	#Wk5 (D8±1 post boost)	Wk6 (D43 ±2)	Wk8 (D57 ±2)	Wk12 (D85 ±3)	Wk24 (D169 ±5)
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Informed Consent	X									
Medical history	X	X								
Demographics	X									
Concomitant Medications	X	X	X	X	X	X	X	X	X	X
Physical Exam[1]	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X[2]	X	X	X[2]	X	X	X	X	X
12-lead ECG	X	X[3]			X[3]					
Hematology	X	X	X		X	X		X	X	X
Chemistry[4]	X	X	X		X	X		X	X	X
Urinalysis	X	X	X		X	X		X	X	X
Serology[5]	X									
Nasopharingeal swab for SARS-CoV-2		X[6]			X[7]					
SARS-COV-2 serology (quantitative)	X									
Pregnancy test[8]	X	X[9]			X[9]			X	X	X
Binding antibodies[10]		X		X	X			X	X	X
Neutralizing antibodies[10]		X			X			X		X
Cellular immune response[10]		X		X	X			X	X	X
Vaccination		X[11]			X[12]					
Adverse Events	←----------									----------→
Participant Diary		X[13]	X[14]		X[13]	X[14]				
Telephone contact		X[15]			X[15]					

#Visit not applicable to subjects belonging to prime group

[1] Full physical examination at screening and last study visit only; targeted examination at other visits, as determined by Investigator or per participant complaints

[2] Vital signs to be performed pre- and 30-min-post- vaccination in subjects undergoing vaccine administrations, at any time for the others

[3] One (1) hour after vaccine administration

[4] Sodium (Na), potassium (K), glucose, BUN or urea, Cr, total bilirubin, ALT, AST, CPK, total proteins, CRP

[5] HIV antibodies, HBsAg, HCV antibodies

[6] Nasopharingeal swab to be performed in the 72 hours before vaccination on D1

[7] Nasopharingeal swab to be performed in the 72 hours before vaccination on D29 for PB groups only

[8] Serum/Urine pregnancy test

[9] On D1 before vaccine administration for all subjects; on D29 before vaccine administration for PB groups, at any time for the others

[10] Details on timing and volumes required for the immunogenicity assessments are provided in section 10

[11] Vaccine administration for all participants

[12] Vaccine administration for participants belonging to PB groups only

[13] Diary hand-over and training on use to participants

[14] Diary collection

[15] In the evening of the day of vaccination and 24 hours after vaccination

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Full name
ACE2	Angiotensin-Converting Enzyme 2
ADE	Antibody-Dependent Enhancement
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical
BGH	Bovine Growth Hormone
CI	Confidence Interval
CMV	Cytomegalovirus
CNS	Central Nervous System
Col E1 Ori	Colicin E1 origin
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus disease 19
CPK	Creatine phosphokinase
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-reactive protein
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CTD1	Chromatin licensing and DNA replication factor 1
CTFG	Clinical Trial Facilitation Group
CTLs	Cytotoxic T-Lymphocytes
DNA	Deoxyribonucleic Acid
EC(s)	Ethics Committee(s)
eCRF	electronic Case Report Form
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EP	Electroporation

Abbreviation or term	**Full name**
FDA	Food and Drug Administration
FVFP	First Visit First Patient
FVLP	First Visit Last Patient
GCP	Good Clinical Practice
GMFR	Geometric Mean Fold Rise
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICH	International Conference for Harmonisation
ICS	Intracellular Cytokine Staining
IDSMC	Independent Data Safety Monitoring Committee
IFN-γ	Interferon-gamma
IMP	Investigational Medicinal Product
IM	intramuscular
INMI	Istituto Nazionale per le Malattie Infettive (National Institute for Infectious Disease)
IntA	Intron A
ITT	Intention-to-Treat
LVLP	Last Visit Last Patient
mAb	Monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
MERS	Middle East Respiratory Syndrome
N protein/N	Nucleocapside protein
nCOV	Novel Coronavirus
PB	Prime-Boost
PBMC(s)	Peripheral Blood Mononuclear Cell(s)

Abbreviation or term	**Full name**
PI	Principal Investigator
PP	Per Protocol
P	Prime
PT	Preferred Term
QC	Quality Control
QP	Qualified Person
RBD	Receptor Binding Domain
RNA	Ribonucleic acid
S protein/S	Spike protein
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SmPC	Summary of Product Characteristics
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
Th-1/Th1	T helper type 1
TMF	Trial Master File
tPA	tissue Plasminogen Activator
V	Volt
WHO	World Health Organization

1. INTRODUCTION

1.1 Background information and rationale

1.1.1 *SARS-CoV-2 – COVID-19*

In December 2019, a pneumonia outbreak of unknown cause occurred in Wuhan, China. Clinically, patients presented with fever, cough, fatigue, anorexia and myalgia, i.e. with symptoms very similar to other respiratory virus infections. In addition, some patients were reported to have radiographic multifocal ground-glass opacities, even those with mild disease and normal or lower than average white blood cell lymphocyte and platelet counts. The disease was characterized by an efficient person-to person transmission, with multiple clusters reported. By January 2020 coronavirus RNA was identified in some of these patients, and this novel coronavirus identified as the etiologic agent is now named SARS-CoV-2 (due to its similarity to the Severe Acute Respiratory Syndrome Coronavirus, SARS-CoV). It has 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV.[1]
Within a month, the genetic sequence of the virus became available (MN908947.3)[2]. SARS-CoV-2 infections and the resulting disease, designated as coronavirus disease 2019 (COVID-19), have spread globally. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic[3], which has rapidly progressed. In just a few months, since December 2019, COVID-19 has spread worldwide with over 25,298,875 confirmed cases and more than 847,602 confirmed deaths world-wide, including 268,218 confirmed cases and more than 35,477 confirmed deaths in Italy, as of September 1st.[4]

1.1.2 *COVID-eVax*

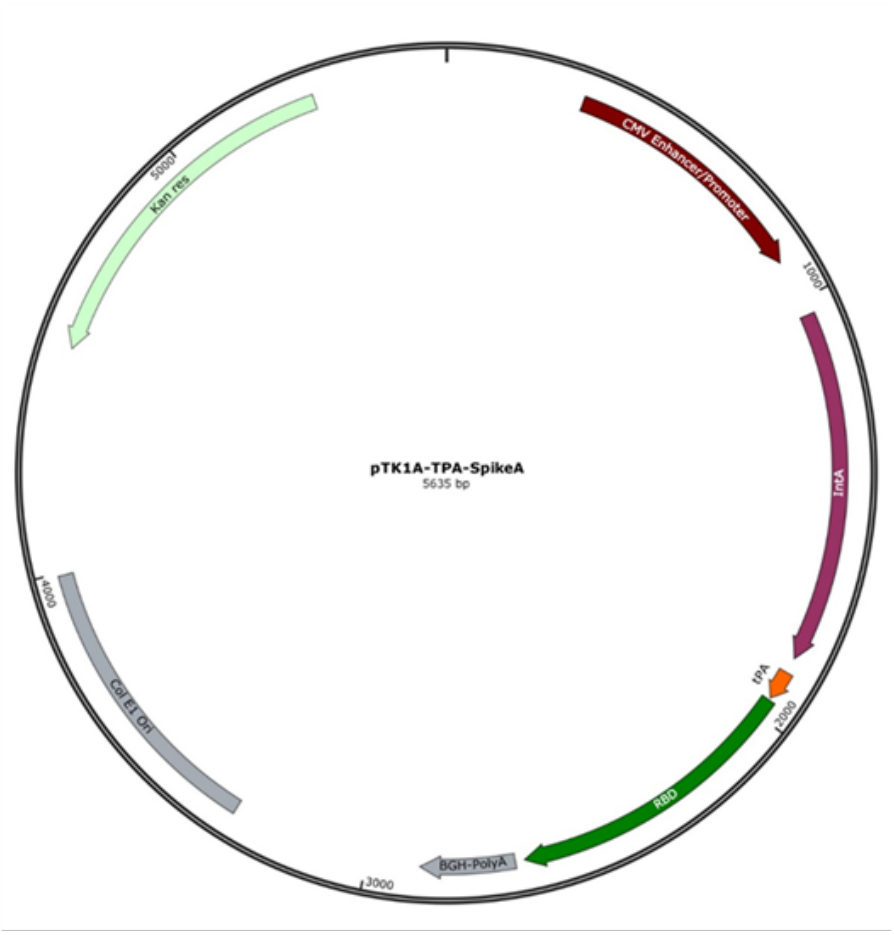
To date, no vaccines against any human-infecting coronaviruses have been approved and due to the devastating characteristics of the COVID-19 pandemic, a vaccine is urgently needed. Several vaccines using different technology platforms are under development with an unprecedented effort to obtain safe and effective prophylactic weapons against COVID-19 infection, which represents an urgent and, so far, unmet public health need.[5] At least 200 research groups have ongoing COVID-19 vaccine development programs, with more than 40 candidate vaccines, based on different technological platforms (e.g. mRNA, DNA, inactivated, protein subunit, replicating or non-replicating viral vector), reported to have started clinical development. A complete and continually updated list is available from the WHO web site[6]. Encouraging preliminary results on the reactogenicity, safety and immunogenicity in humans are already available for several candidate COVID-19 vaccines (see https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/ for a continually updated summary of the available clinical trial data).

DNA-based platforms are among those with the greatest potential for safety and speed in development, both critical to contrast a pandemic such as COVID-19 and, potentially, future pandemics.

COVID-*e*Vax, the candidate vaccine to be tested in the present study, is a DNA plasmid-based vaccine which will be administered by intramuscular (IM) injection with electroporation (EP) applied to the injection site. The target antigen of COVID-*e*Vax, namely Spike A, is the Receptor Binding Domain (RBD) portion of the SARS-CoV-2

Spike protein (comprised between residues 319-541, Wuhan strain NC_045512.2) fused with a tissue Plasminogen Activator (tPA) leader sequence. The tPA leader sequence allows proper secretion of the antigen. Schematic representations of COVID-*e*Vax and of SARS-CoV-2 S protein are shown in Figure 1 and Figure 2, respectively.

Figure 1. Schematic Representation of COVID-*e*Vax



Description of the elements

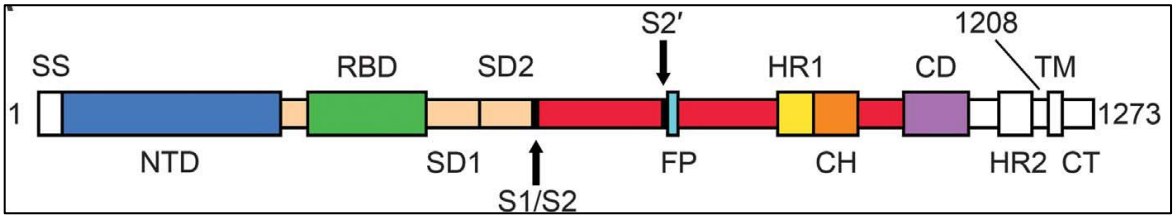
Plasmid Backbone

- ColE1 Ori: ColE1 Origin for plasmid replication
- Kan res: Kanamycin Resistance Gene for clone selection

Expression Cassette

- CMV Enhancer/Promoter: Human cytomegalovirus (CMV) immediate-early promoter/enhancer driving gene expression
- IntA: intron A from Human cytomegalovirus (CMV) for mRNA stabilization
- tPA: leader sequence for protein secretion
- RDB: RBD of Spike protein of SARS-CoV-2 virus, the antigen of interest
- BGH-Poly A: Bovine growth hormone (BGH) polyadenylation signal as terminator of Transcription

Figure 2. Schematic Representation of SARS-CoV-2 Spike Primary Structure coloured by Domain



Note:
Domains that were excluded from the ectodomain expression construct or could not be visualized in the final map are colored white.
SS, signal sequence; S2′, S2′ protease cleavage site; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail.
Arrows denote protease cleavage sites.[7]

COVID-*e*Vax was selected among 5 different candidates, encoding the full-length protein, portions of it and engineered variants. Based on the pre-clinical results, it has been observed that all variants were capable of generating antibodies against the RBD region. In particular, the construct encoding for Spike A gave the greatest antibody titer and was equally effective as the construct encoding for the full length S protein in the neutralizing assay with pseudotyped-viruses.

Key features and advantages of COVID-*e*Vax are as follows:

1. COVID-*e*Vax provides the important advantage of being able to be administered several times, without the induction of neutralizing antibodies against the vaccine itself, as instead may happen in the case of virus-based vector vaccines;

2. For the same reason, it can be used as a booster e.g. to viral vector vaccines that may not be repeatable in case of short-lasting immunity;

3. COVID-*e*Vax is a highly efficient vaccination platform capable of inducing both antibodies and a Th-1 cell-mediated response;

4. SARS-CoV-2 may acquire mutations capable of substantially changing its pathogenicity. COVID-*e*Vax targets the RBD region which is very conserved among emerging variants,[8] it is key for viral infection and antibodies binding to this region have been shown to efficiently neutralize the virus;

5. Nevertheless, in case of mutations of the virus sequences, a specific genetic design can be easily adapted to COVID-*e*Vax (i.e. it is possible to insert new sequences identified by epidemiological analysis into the vaccine) to target SARS-CoV-2 in specific geographical regions or the mutated version of the virus globally;

6. COVID-*e*Vax is supposed to minimize the risk of inducing an Antibody-Dependent Enhancement (ADE) effect thanks to the fact that it targets a minimal region within

Spike protein (RBD): in coronaviruses, ADE can be promoted by suboptimal antibodies to the full-length Spike protein[9];

7. COVID-*e*Vax does not require complex formulations such as lipoparticles (for peptides or RNA vaccines) or other nanoparticles (for other vaccines). The plasmid DNA is produced in bacteria and then extracted and purified, without using viral vectors amplified in mammalian cells. This allows shorter production times, less expensive process and easier transport and storage;

8. COVID-*e*Vax is made of DNA, a molecule potentially stable for long time at Room Temperature. In future, this would allow shipment and storage without a cold chain, a unique advantage compared to the other vaccine platforms;

9. Finally, the DNA-technology may assure a longer duration of gene expression and thus immunization, and could be used in several other areas of prevention and therapeutics.

COVID-*e*Vax was validated in pre-clinical settings with the support of the Spallanzani Institute (INMI – Istituto Nazionale Malattie Infettive) and of the University of Ulm, confirming that the sera of the vaccinated animals are able to block the infectivity of the virus *in vitro* on cells.

Detailed information on the preclinical experiments performed so far are provided in the COVID-*e*Vax Investigator Brochure[10].

1.1.3 *Human experience with DNA plasmids for gene expression*

Multiple studies have reported that DNA vaccines allow for the generation of cellular and humoral responses against pathogens. Clinical studies using DNA-based platform have been recently reviewed by Gary and Wiener, who convincingly show how the improvement in DNA-based platforms make them ideal for rapid vaccine development against emerging infectious disease.[11] In this section we focus on 3 DNA vaccines targeting coronavirus S proteins that have been already tested in humans.

The first candidate DNA vaccine expressing SARS S protein was VRC-SRSDNS015-00-VP and was tested in 10 healthy adults, aged 21 to 49 years, in 2004 and 2005 following a rapid vaccine development response to the SARS outbreak.[12] VRC-SRSDNS015-00-VP DNA vaccine at a dose of 4 mg was administered IM by a Biojector needle free device according to a prime-double-boost dosing schedule at Time 0 (baseline), Week 4 and Week 8. The vaccine was safe and well tolerated. Local and systemic reactogenicity events were mild and transient. There were no SAEs and no grade 3 or 4 AEs. Following the first vaccination, the SARS candidate vaccine was immunogenic as assessed by ELISA and pseudotyped lentiviral vector neutralization assay in most subjects with peak response after the 3rd vaccination. Vaccine-induced T cell responses were detected in all subjects.

Another candidate DNA vaccine expressing MERS S protein was GLS-5300, evaluated in 75 healthy subjects, aged 19 to 50 years, in 2016.[13] GLS-5300 DNA vaccine was administered IM followed by EP according to a prime-double-boost dosing schedule at Time 0, Week 4 and Week 12, in a dose escalation trial at a dosage of 0.67, 2 or 6 mg. Overall, the vaccine was safe and well tolerated. Local and systemic reactogenicity events were generally mild and transient. There were no SAEs and no grade 3 or 4 AEs attributed

to vaccination, except for one severe induration following the third vaccination resolving in 24 h. The MERS candidate vaccine was immunogenic as assessed by seroconversion and vaccine-induced T cell responses in most vaccine recipients.

The third candidate DNA vaccine, INO-4800, is probably the most relevant as it expresses the SARS-CoV-2 S protein. This vaccine is being evaluated in 40 healthy subjects, aged 18 to 50 years, at doses of 1 or 2 mg. Administration is by intradermal injection followed by EP using Inovio's Cellectra® device according to a prime-boost dosing schedule at Time 0 and Week 4. According to a company's press release, INO-4800 was generally safe and well-tolerated in all participants in both cohorts through Week 8. There were no reported SAEs. All reported AEs were grade 1 in severity, and most were local injection site redness. Only preliminary immunogenicity data after two doses at Week 6 in most vaccinated recipients have been reported. Immunogenicity was assessed by multiple humoral and cellular immune responses: 94% of participants demonstrated both humoral (binding and neutralizing antibody) and T cell immune responses, indicating that the COVID-19 candidate vaccine INO-4800 was immunogenic.

1.1.4 *DNA Electroporation Technology*

Originally, IM inoculation using highly concentrated formulations, have induced consistent, albeit small, immunological responses of DNA-based vaccines. An increased DNA uptake can be obtained with different methods.[14] Among others, EP[15] increases the initial uptake of DNA plasmid by local cells approximately 500 folds over needle and syringe delivery only.

Following local injection by needle and syringe only, plasmid DNA is taken up by a limited number of cells at the site of injection, where the DNA is transcribed into mRNA and translated into antigen intracellularly. When the IM inoculation is associated to EP, that consists in the application of an electrical field to the cells in order to increase the permeability of the cell membrane, the transport of large and highly charged molecules (such as DNA) across the hydrophobic bilayer membrane is highly facilitated.[16,17]

EP demonstrated to be useful to increase the effectiveness of DNA vaccines in different species (such as mice, rabbits and guinea pigs) including humans. The first medical application of EP was for delivering poorly permeant anticancer drugs into tumor nodules[18]. Thereafter, plasmid DNA (pDNA)-based gene transfer has emerged also as an attractive platform to target infectious disease[19,20,21,22,23,24].

Takis's scientists have explored several electrical conditions in preclinical models (mice, rats, rabbits, dogs, nonhuman primates) generating data which allowed the execution of several clinical trials with different EP technologies by other companies (e.g. Merck with Inovio technology[25,26,27] and by Johnson&Johnson with the ICHOR device[28]). Takis adopted an EP Technology manufactured by IGEA, an Italian company leader in tissue EP. The IGEA proprietary device is able to deliver both High Voltages (100-1000 V) with pulses length from 50-1000 μsec and Low Voltages ranging from 20 to 200 V and pulses lengths of 1-200 msec. This device has been extensively tested both in mice and rhesus monkeys and the results have shown that it is highly efficient in mediating gene transfer of plasmid DNA, thus allowing the induction of measurable immune responses to the target antigens. Moreover, its use in humans, in addition to many applications for Electro-Chemo-

Therapy approved by EMA, was also approved by the Istituto Superiore di Sanità in 2011 for a clinical trial aimed at assessing a DNA vaccine to be delivered intramuscularly followed by EP targeting ErbB2 (RHuT-IDN6439) (EudraCT: 2011-001104-34, protocol code: IOV-HN-1-2011).

All the DNA-EP data with COVID-*e*Vax have been obtained using the IGEA proprietary technology, the same that is going to be utilized for the present clinical trial.

Overall, there is substantial evidence that EP after IM injection of the DNA vaccine can be considered safe and well tolerated. The safety and tolerability of IM injection of saline followed by EP using the Cellectra® device (Inovio), similar to the IGEA's one, are available from two open-label studies in healthy adults[29]. Local pain was present but subsided quickly, and most related AEs were mild injection site reactions. The VAS pain score peaked immediately after EP but declined to about 50% within 5 min for the majority of subjects (more details on the AEs reported can be found in Table 1.4, at page 23-24 - study protocol GLS-5300 -Dated 13Nov2017, v10.0).[30]

In conclusion, the results available from the growing number of clinical trials indicate that DNA EP combines increased efficiency with safety. Studies using DNA plasmids-based vaccines similar to COVID-*e*Vax indicate that the administration procedure, consisting of IM injection followed by EP, can also be considered safe and tolerated, eliciting local and systemic effects with a general pattern similar to the one observed with other vaccine administration procedures not including EP.

1.2 Risk-benefit assessment of the study

The present open label Phase I/II study is designed to assess the safety, reactogenicity, and preliminary immunogenicity of COVID-*e*Vax. The clinical hypothesis is that the IM administration of the DNA plasmid encoding for the RBD of the SARS-CoV-2 spike protein, followed by EP, will induce a humoral and cellular immune response which will generate levels of neutralizing antibodies and cytotoxic T-cells as a potential prophylactic treatment for SARS-CoV-2 infection.

This Phase I/II study is the first clinical trial with COVID-*e*Vax. There are no safety data in humans on COVID-*e*Vax administration yet and the trial has been designed to minimize risks to study participants. COVID-*e*Vax will be initially administered starting with the lowest dose and utilizing a cohort progression scheme consisting of sentinel/ dose staggering techiques before progressing to the higher doses (see Section 3.1).

The potential risks to participants are those commonly associated with vaccination.

1.2.1 *Potential risks*

Systemic and local reactions to vaccination

Systemic side effects with vaccines based on similar DNA plasmids-based vaccines have been demonstrated to be generally minimal. These might include a flu-like syndrome, with or without fever, fatigue, malaise, arthralgia, myalgia and headache.

As with any new product, there is the potential risk of a serious, even life-threatening, allergic reaction also with the COVID-*e*Vax administration. Anaphylaxis is an extremely

rare event (occurring 1 in about 1,000,000 vaccine doses), but can occur in response to any vaccine or medication. To mitigate this risk, potential participants with a history of severe allergic reaction of any kind or allergic reaction to any vaccine, will be excluded from participation. As the vaccine is formulated in Dulbecco's phosphate-buffered saline and will be diluted in saline, the allergic reaction would most likely be due to the DNA vaccine. In the event of a severe allergic reaction, trained medical personnel with appropriate medical emergency equipment to provide acute care for conditions such as anaphylaxis will be available in close proximity to the trial site where, if required, emergency departments capable of treating serious adverse reactions, will be available.

Local reactions to vaccination might include injection site pain, tenderness, erythema, induration or swelling, bruising and pruritus.

As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage may occur, but this should be extremely rare.

The genetic risk due to the possible integration into the host genome of COVID-eVAX plasmid DNA following intramuscular delivery and electroporation is considered to be extremely low. This speculation is supported by biodistribution studies that excluded that COVID-eVax plasmid DNA persists in any tissue, including the site of injection, at level exceeding 30,000 copies per ug i.e. the threshold above which, according to current regulatory guidelines, there is the risk of integration[31].

Disease Enhancement (Antibody-Dependent Enhancement, ADE)

Safety concerns consisting in an enhancement of the disease upon infection with SARS-CoV-2 following the use of investigational candidate vaccines have been raised. Historical and limited reports of immunopathology and ADE were reported in vitro and post SARS-CoV challenge in mice, ferrets and non-human primates immunised with whole SARS-CoV inactivated or full-length S protein based vaccines.[9] This includes a study using Modified Vaccinia Ankara as a vector.[32,33,34] To date, there has been one report of lung immunopathology following MERS-CoV challenge in mice immunised with an inactivated MERS-CoV candidate vaccine.[35]

The risk of ADE following the administration of COVID-*e*Vax should be minimal if not absent due to the peculiar vaccine construct targeting the RBD of the S protein only: in coronaviruses, ADE can be promoted by suboptimal antibodies to the full-length Spike protein[9]. The construct is meant to maximize the induction of properly neutralizing antibodies and of Th1 skewed immune responses that are known to prevent or blunt inflammatory and allergic immune responses. A detailed analysis of the reasons why the risk of ADE is negligible with COVID-*e*Vax can be found in the product Investigator Brochure[10]. However, efficacy studies in ACE2 mice, ferrets, and cats infection models are underway to assess the presence or absence of lung pathology. Results will be reviewed as soon as they emerge and will inform on the potential risk to participants receiving COVID-*e*Vax. All pathology data arising from challenge studies of other SARS-CoV-2 vaccine candidates will also be taken into account.

As a risk mitigation strategy, during the post-vaccination follow up, extra-visits are foreseen in case any participant develops febrile or respiratory symptoms (as described more in details in Section 11.4.2). If the Investigator considers these symptoms compatible

with COVID-19, the participant will be assessed for the presence of SARS-CoV-2 infection in respiratory tract samples. This measure is meant to ensure that potential exposure to SARS-CoV-2 in vaccinated participants will be properly monitored, to assess as early as possible the occurrence of any sign or symptom of a vaccine-mediated disease enhancement.

This potential risk will also be clearly stated in the Informed Consent Form.

Phlebotomy

Some localized discomfort can occur at the site of venipuncture for the blood draw, including swelling, local tenderness or bruising.
Rarely, infection at the site of venipuncture may develop and a few participants may feel light-headed and may develop tachycardia during blood collection. These symptoms can be stopped by having the participant lie down and/or by stopping the procedure. Should these rare events occur, they can be easily treated as per standard practice.

Due to the nature and design of the study requiring frequent blood sampling, up to 600mL of blood will be withdrawn (blood volumes may vary slightly at different investigator sites due to the use of different volume vacutainers, and will also depend on how long the participants will be retained in the study) over the duration of the study. This will not compromise the health of the study participants as blood donors donate about 500mL during a single blood donation session (every 3-4 months). For precaution, study participants will be asked to refrain from blood donation for the duration of their involvement in the study.

Reactions to electroporation (EP)

Minor potential risks relate to the EP procedure used in this study to facilitate entry of the DNA into the target site (muscle cells). The EP procedure delivers short lasting trains of electrical pulses through 4 electrodes inserted into the deltoid muscle. The electrical field generated by the EP may result in a brief but intense muscle contraction with each pulse, which may be associated with pain and tenderness at the injection site.[29] Rarely, minor transient cutaneous bleeding at the sites of needle and electrode penetration have been reported after IM injections.[30]

Asymptomatic blood creatine phosphokinase (CPK) level increase due to transient muscle damage has been observed after intramuscular EP.[29] Evaluation of CPK and ECG for cardiac conduction abnormalities have been included in this study.

Pregnancy

During the entire study, and for a period of at least 90 days after the last COVID-*e*Vax administration in case of patient withdrawal, participants of childbearing potential must use a highly effective method of birth control (see Section 11.5).

Unknown Risks

As with all research there is the remote possibility of risks that are unknown or that cannot be foreseen based on the current available information.

1.2.2 *Potential benefits*

No direct guaranteed benefit is expected for study participants, except undergoing an accurate check-up about their general health status at no costs. It is hoped, however, that the information gained from the study will contribute to the development of a safe and effective vaccine against COVID-19, which will be beneficial for the community. Should the vaccine be able to generate protective levels of neutralizing antibodies and cytotoxic T-cells, study participants may be potentially protected against SARS-CoV-2 infection, although the duration of such protection is unknown.

1.2.3 *Conclusion*

In conclusion, even in the absence of direct guaranteed clinical benefits for study participants associated with the proposed preventive treatment, in consideration of the overall safety profiles of similar DNA plasmid-based vaccine, the benefit–risk ratio of the proposed study is believed to be favourable.

2. STUDY OBJECTIVES

2.1 Phase I (Dose Escalation)

2.1.1 *Primary objective*

- To assess the safety and reactogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To identify the dose(s)/schedule(s) to be used in the Phase II (Dose Expansion)

2.1.2 *Secondary objectives*

- To preliminarily assess the immunogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

2.2 Phase II (Dose Expansion)

2.2.1 *Primary objective*

- To assess the immunogenicity of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

2.2.2 *Secondary objective*

- To assess the duration of the immune response of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To assess the (long-term post-administration) safety of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

3. OVERALL STUDY DESIGN AND PLAN

3.1 Study design, schedule, and chronology

This is a multicentre, open-label Phase I/II study, with a first-in-human (FIH) dose escalation part (Phase I study) followed by an open-label single arm (or two-arm, randomized) dose expansion part (Phase II study) in males (M) and non-pregnant females (F), in a 1:1 M/F ratio within each cohort, aged 18 to 65 years, who are in good health and meet all eligibility criteria.

Overall Description of the Study Methodology

The Phase I part of the study will investigate the safety and preliminary immunogenicity of ascending doses (in terms of unit dose and dose schedule) of COVID-*e*Vax, to inform the decision to move to Phase II.

The Phase II part of the study will consist of the expansion of 1 or 2 dose(s)/schedule(s) (the most promising), to primarily confirm immunogenicity and expand safety data of COVID-*e*Vax in a larger healthy subject population.

COVID-*e*Vax will be administered both as prime-boost and prime only schedules by the IM route, followed by EP applied through a commercially available Electro Gene Transfer (EGT) device.

The study will begin with an open-label dose escalation in sequential cohorts (Phase I). Three escalating cohorts of 20 subjects each are planned to be enrolled sequentially, as follows:

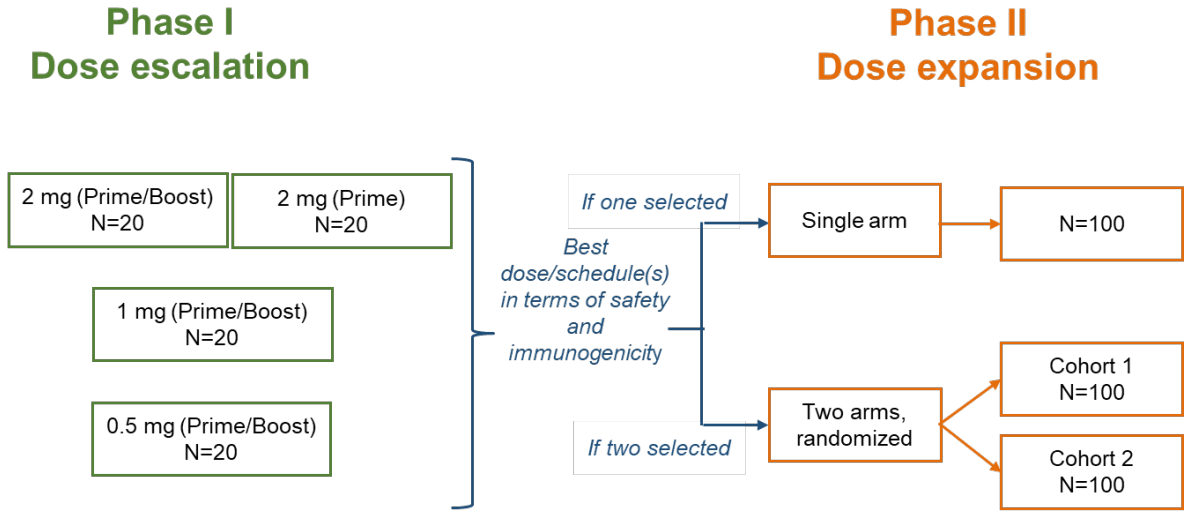
- Cohort 1: 0.5 mg prime and 0.5 mg boost (PB), 4 weeks apart - Total dose: 1 mg
- Cohort 2: 1 mg prime and 1 mg boost (PB), 4 weeks apart - Total dose: 2 mg
- Cohort 3: 2 mg prime and 2 mg boost (PB), 4 weeks apart - Total dose: 4 mg

A further group is planned, (starting after completion of sentinel subjects of cohort 3), to test the prime schedule:

- Cohort 4: 2 mg prime (P) - Total dose: 2 mg

A schematic overview of the study design is presented in Figure 3.

Figure 3. Overview of the study design



The 100 subjects/cohort of the Phase II include the 20 subjects who received the same dose schedule in Phase I.

Participants will undergo 2 (4 weeks apart) or 1 only vaccination sessions depending on the assigned dosing schedule, prime-boost (PB) or prime (P) respectively.

The Phase I - Dose escalation part of the study encompasses a total of 12 (11 for P subjects) clinic visits:

Screening Visit (V1)
Baseline Visit (V2 - D1, 1st vaccination administration)
Day 3 (V3)
Week 1 (V4)
Week 2 (V5)
Week 4 (V6); D29 - 2nd vaccination administration, not applicable for P subjects
Week 5 (V7), 1 week after boost dose, visit not applicable for P subjects
Week 6 (V8)
Week 8 (V9)
Week 12 (V10)
Week 16 (V11)
Week 24 (V12, Final Visit)

The Phase II - Dose expansion part of the study encompasses a total of 10 (9 for P subjects) clinic visits:

Screening Visit (V1)
Baseline Visit (V2 - D1, 1st vaccination administration)
Week 1 (V3)
Week 2 (V4)
Week 4 (V5) - D29 - 2nd vaccination administration, not applicable for P subjects
Week 5 (V6), 1 week after boost dose, visit not applicable for P subjects
Week 6 (V7)

Week 8 (V8)
Week 12 (V9)
Week 24 (V10, Final Visit).

The detailed schedule of evaluation, by visit, is displayed in the study flow-chart (see Section 10).

Experimental Procedure:

The Phase I portion of the study will follow a cohort progression scheme, to assess and ensure the safety of each vaccine dose schedule, by sentinel dosing/dose staggering techniques, ensuring appropriate interval(s) between dosing of the first subjects within a cohort and enrolment of the remainder of participants in the same cohort (as well as into the next higher dose schedule cohort), that will be opened only after review of the safety and tolerability data of the 6 sentinel participants in that cohort.

Dosing will begin with 6 participants in the lowest cohort (Cohort 1 – 0.5 mg, prime boost), starting with the 1st sentinel participant.
Local and systemic AEs occurred and lab examinations performed 48 hours after the vaccine administration will be reviewed and, if no safety concerns emerge, the 2nd and 3rd sentinel participant of this cohort will be enrolled, 3 days after enrolment of the 1st sentinel Cohort 1 participant. The same applies to the 4th, 5th and 6th sentinel participant, who will be enrolled, in case of no safety concerns in the first 3 subjects, 3 days after enrolment of the 2nd and 3rd sentinel participant.

The same applies to the remaining 14 participants in cohort 1, who will be enrolled, in case of no safety concerns in the first 6 subjects, 3 days after enrolment of the 6th sentinel participant.

The next cohort can be opened after AEs and clinical laboratory data review of all participants in the previous cohort for the 7-day period, ideally 8 days after the last subject in the previous cohort was treated.

The same sentinel dosing/cohort completion scheme will be followed also for Cohorts 2 and 3; Cohort 4 can be started after completion of sentinel subjects in Cohort 3.

Vaccine administration will be performed under strict sterile conditions. After the vaccine administration the study participants will remain at the clinical site for observation, in case of immediate adverse events, for at least 4 hours. At discharge, participants will be provided with a thermometer, a ruler and a diary with instructions on use, along with the 24-hour emergency telephone number, to make him/her able to contact the study physician, if needed.

After each vaccination, all participants will be contacted by telephone in the evening of the day of vaccination and 24 hours after vaccination, to assess whether there are any local or systemic reactions and if an unscheduled visit is needed.

Thereafter, all participants will be monitored for solicited and unsolicited local and systemic AEs at each study visit.

Reactogenicity will be measured by the incidence of solicited local AEs at injection site and solicited systemic AEs from the time of each vaccination through 7 days post vaccination.

Clinical safety laboratory evaluations will be performed at screening, prior to first vaccination, 2 days after vaccination, and then at Week 1, 4 (D29), 5, 8, 12 and 24 post the first vaccination. For subjects belonging to prime-boost groups, laboratory evaluations scheduled the day of second vaccination (Week 4 - Day 29) should be performed before the vaccination. Laboratory evaluation at week 5 (1 week after boost vaccination) won't be applicable for subjects belonging to prime group.

Safety data will be reviewed by the Independent Data Safety Monitoring Committee (IDSMC) on a regular basis, until all participants have completed the study (see Section 14.1).

Immunogenicity evaluation will consist in the assessment of humoral and cellular immune responses. Humoral immune response evaluations will include quantitation of anti-S and anti-N antibodies and SARS-CoV-2 neutralizing antibodies at multiple timepoints post each vaccination, as measured by ELISA and live virus neutralization assays. Cellular immune evaluation will consist in characterizing T cell responses to SARS-CoV-2 proteins in peripheral blood mononuclear cells (PBMCs).

All immunogenicity evaluations will be performed by a central laboratory.

Phase II will start after reviewing the reactogenicity and immunogenicity data obtained at 4 weeks after the last vaccination in all participants enrolled in Phase I, i.e. when the participants belonging to P group have completed the visit scheduled 4 weeks after the vaccine administration and the participants belonging to PB groups have completed the visit scheduled 4 weeks after the 2nd vaccine administration.

The best dose/schedule in terms of safety and preliminary immunogenicity will be selected for the dose expansion part of the study (Phase II). Two doses/schedules may be selected, in case of a minimum difference between the best two.

Phase II has been designed as an open-label single arm (or randomized two-arm) study, in healthy volunteers. Additional participants will be enrolled to reach a sample size of 100 subjects in the expanded dose schedule cohort(s). Therefore, if only one dose/schedule is selected for expansion, 80 subjects (in addition to the 20 enrolled in the corresponding Phase I cohort) will be enrolled, while, if two doses/schedules are selected for expansion, 160 additional subjects will be enrolled.

Enrolment in phase II will not follow the sentinel dosing/dose staggering. The schedule of clinic visits and assessments will replicate the one of Phase I, excluding the visits scheduled 48 hours and 16 weeks after the first vaccination, which are applicable to Phase I only.

3.2 Study Endpoints

3.2.1 *Phase I - Primary endpoint*

Safety

- Reactogenicity:
  - Incidence of solicited local AEs at the injection site *(time frame: through 7 days post-each vaccination)*
  - Incidence of solicited systemic AEs *(time frame: through 7 days post-each vaccination)*

- Incidence of unsolicited AEs *(time frame: through 4 weeks post-each vaccination)*

- Changes in safety laboratory parameters *(time frame: through 4 weeks post-each vaccination)*

3.2.2 *Phase I - Secondary endpoints*

Immunogenicity
(time frame: through 4 weeks post-last vaccination)

- Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen, analysed as Geometric Mean Titer (GMT) and Geometric Mean Fold Rise (GMFR) from baseline
- SARS-CoV-2 neutralizing antibody titer, analysed as GMT and GMFR from baseline
- Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2 as determined by Interferon-gamma (IFN-γ) ELISpot
- Percentage of subjects who seroconverted
- Duration of the immune response on all criteria and parameters used as secondary endpoint *(time frame: through study completion)*

Safety

- Incidence of unsolicited AEs through study completion (*6 months*)

All primary and secondary endpoints will also be assessed in the time frame through study completion (6 months).

3.2.3 *Phase II - Primary endpoints*

Immunogenicity
(time frame: through 4 weeks post-last vaccination):

- Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen, analysed as GMT and GMFR from baseline

- SARS-CoV-2 neutralizing antibody titer, analysed as GMT and GMFR from baseline
- Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2 as determined by IFN-γ ELISpot
- Percentage of subjects who seroconverted

3.2.4 *Phase II - Secondary endpoints*

Immunogenicity
(time frame: through study completion at 6 months post-first vaccination):

- Duration of the immune response on all criteria and parameters used as primary endpoint

Safety

Reactogenicity:
- Incidence of solicited local AEs at the injection site
  (time frame: through 7 days post-each vaccination)
- Incidence of solicited systemic AEs
  (time frame: through 7 days post-each vaccination)

- Incidence of unsolicited AEs
  (time frame: through 4 weeks post-each vaccination)

- Changes in safety laboratory parameters
  (time frame: through 4 weeks post-each vaccination)

- Incidence of unsolicited AEs through study completion (6 months)

All primary and secondary endpoints will also be assessed in the time frame through study completion (6 months).

3.3 Study duration and key decision points

The study is planned to start in December 2020.

Key decision points

- Availability of safety and immunogenicity data (4-week) for Phase I and decision to move to Phase II: March 2021
- Availability of safety and immunogenicity data (4-week) for Phase II and decision to move to Phase III: June 2021

Study completion will occur when all subjects have completed the 6-month observation period (November 2021).

For a single subject the study will last up to 7 months.

4. JUSTIFICATION OF STUDY DESIGN

4.1 Study design

This Phase I/II study has been designed as a multicentre, open label, with a first-in-human non-randomized dose escalation part (Phase I) followed by a (possibly randomized) dose expansion part (Phase II), adopting a scheme similar to the one in use in other ongoing early phase clinical trials aimed at assessing the tolerability, reactogenicity and immunogenicity of candidate vaccines against SARS-CoV-2.

This clinical trial will evaluate whether COVID-*e*Vax administered via IM injection and followed by EP is able to generate immunity against SARS-CoV-2 and if the response is dose related.

The best dose schedule (or the best two dose schedules if there is a minimal difference between the two best doses) in term of safety and immunogenicity in Phase I will be selected for expansion in Phase II, with the purpose of generating additional and more robust safety and immunogenicity data to inform the choice of the dose schedule to be evaluated in Phase III.

This study is designed as an open-label study, without a placebo arm. Given the small sample size and the route and procedure of administration, the use of a placebo group is unlikely to improve understanding of the pattern of elicited AEs. In addition, the evaluation of immunogenicity is based on objective and quantitative laboratory measurements, making unlikely that the study results can be significantly biased. Finally, an unblind study will facilitate the need for rapid review and decision making.

4.2 Appropriateness of Measurements

The clinical and laboratory safety and immunogenicity measurements used in this study are in line with the most recent EMA "*Guideline on Clinical Evaluation of New Vaccines*"[36], the FDA "*Development and Licensure of Vaccines to prevent COVID-19 - Guidance for Industry*"[37], and the state of the art of early studies investigating the effects of candidate vaccines against SARS-CoV-2.

As far as the safety assessment is concerned, solicited adverse events will be scored based on scales derived from the FDA guidance "*Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*"[38].

Indicators of reactogenicity will include solicited local and systemic AEs. Unsolicited AEs and routine laboratory safety assessments will also be collected and assessed through the study.

As far as immunogenicity assessments are concerned, both humoral (binding and neutralizing antibody titers) and cell-mediated (total $CD4^+$ and $CD8^+$ T-cell responsiveness to SARS-CoV-2 peptides) immune responses will be assessed.
Measurement of serum neutralizing activity has been shown to be a mechanistic correlate of protection for other respiratory viruses, such as influenza[39] and respiratory syncytial virus,[40] and is generally accepted as a functional biomarker of the in vivo humoral response.[41] Even though correlates of protection from SARS-CoV-2 infection have not yet been determined in humans, in rhesus macaques given DNA vaccine candidates (expressing different forms of the SARS-CoV-2 S protein), post-vaccination neutralizing

antibody titers were correlated with protection against SARS-CoV-2 challenge[42] and humoral and cell-mediated immune responses have been associated with vaccine-induced protection against challenge or subsequent rechallenge after SARS-CoV-2 infection.[43] Binding and neutralizing antibody titers will be also compared to those found in convalescent serum specimens.
The assessment of T-cell immune responses using the IFN-γ ELISpot assay will allow to determine the proportion of Th1 response, with both efficacy and safety implications.

4.3 Suitability of the study population

This phase I/II trial will be conducted in healthy subjects, 18 to 65 years of age, inclusive. In order to take in due account the gender differences in the immune response, males and females will be recruited in a 1:1 ratio within each cohort.

All subjects participating in the study should be healthy on the basis of medical history, physical examination and laboratory tests. In consideration of the more severe forms of COVID-19 observed in presence of comorbidities, a BMI >30, type 2 diabetes or glucose intolerance, hypertension, Chronic Obstructive Pulmonary Disease (COPD), and any cardiac diseases are explicitly listed as exclusion criteria.

Pregnant women are not eligible for the trial. Women of childbearing potential will be required to have a negative pregnancy test immediately prior to each vaccination and to be willing to use a highly effective method of contraception.

Children and elderly will not be included in this trial as presently there are no safety or efficacy data in adults.

Should the outcome of this trial be deemed acceptable, additional trials may be initiated, including subjects in other populations.

4.4 Selection of the doses used in this study

No human trials of COVID-*e*Vax have been conducted to date.

The dose levels of COVID-*e*Vax selected for the present study are 0.5 mg, 1 mg and 2 mg, administered with a prime-boost schedule, and 2 mg administered with a prime schedule, for a total dose ranging from 1 mg to 4 mg.

These dose levels are supported by the results of the completed nonclinical immunogenicity, toxicity and biodistribution studies.
These studies were conducted using the drug product at the same concentration and formulation to be used in the present study. In toxicological studies the product was tested in rats at two doses: the maximum ethically feasible dose (high dose level, 420 μg/rat) and a fraction of it (low dose level, 105 μg/rat). The low dose has been proved to induce an immune response in the rat. The high dose is 1/5th of the highest dose of 2 mg to be used in the first human clinical study. When expressed per unit of body weight, the dose equates approximately to 2 mg/kg, which is 50-fold higher than the highest dose of 0.04 mg/kg (i.e. 2 mg, assuming a 50 kg body weight) and 200-fold higher than the lowest dose to be used in the present study (0.5 mg corresponding to 0.01 mg/kg).

Immunogenicity tests performed in mice showed neutralizing antibodies against SARS-CoV-2 following administration already of 20 μg of DNA. Therefore, the proposed dose levels are within the range of equivalent human doses effective in vaccinated animals.

Detailed information on the preclinical experiments performed so far are provided in the COVID-*e*Vax Investigator Brochure[10].

4.5 Control group

Not applicable, this is an open label, uncontrolled study (see also Section 4.1).

5. STUDY ADMINISTRATIVE STRUCTURE

5.1 Sponsor and Partner

Takis S.r.l. and Rottapharm Biotech S.r.l. entered into an agreement and are collaborating for the co-development of COVID-*e*Vax.

In particular:

Takis S.r.l. is a biotech company specialized in *in vivo* gene transfer, regulation of gene expression and immunogenicity, and is the developer and the owner of the COVID-*e*Vax patent.

Rottapharm Biotech S.r.l. is a research company dedicated to the development of innovative drugs, with experience in preclinical and clinical development. Rottapharm Biotech structure involves all key management functions to support the development of COVID-*e*Vax, including Pharmacology and Toxicology, Pharmacokinetics, Biostatistics, Drug Safety, Clinical Research and Regulatory Affairs.

On this basis:

Takis S.r.l. is the Sponsor of this study and is the ultimate responsible for the quality and integrity of trial data.

Rottapharm Biotech S.r.l. is the Partner, responsible for study design, study management, safety, data interpretation, and writing of the study reports.

The Study Director and medical responsible is Lucio Rovati, M.D. Chief Scientific Officer at Rottapharm Biotech.The activities under the responsibility of Rottapharm Biotech, are being provided according to the relevant tasks and in compliance with all the applicable Rottapharm Biotech's or delegated CRO's SOPs.

5.2 Investigational sites

This is a multicentric study to be conducted in 3 sites, selected in Italy, as follows:

- Istituto Nazionale Tumori, IRCCS, Fondazione G. Pascale, Via Semmola, Naples.
  Principal Investigator: Paolo Antonio Ascierto.
- University of Milano-Bicocca and San Gerardo Hospital, Via Pergolesi 33, Monza.
  Principal Investigator: Paolo Bonfanti.
- INMI Lazzaro Spallanzani, Via Portuense, 292, Roma.
  Principal Investigator: Simone Lanini.

Coordinating Investigator: Paolo Antonio Ascierto.
Co-coordinating Investigators: Paolo Bonfanti and Simone Lanini.

The Investigator/Institution involved in the trial should support Rottapharm Biotech with all documents needed to submit a valid request for authorization to the Competent Authority and the Ethics Committee (EC), and required by GCP. In particular, Investigators' qualification, agreements, and adequate resources will be documented as follows:

- Up-to-date Curriculum vitae (signed and dated) documenting education, training, and relevant experience of the Principal Investigator and all other staff involved at each site
- A list of Investigators' signatures, to document signatures and initials of all persons authorized to take part in the study and their responsibilities
- The Investigator Protocol Agreement Page of this protocol (and any other agreement relevant to trial procedures and obligations), signed and dated by the Principal Investigator.

In addition, the Investigator/Institution should be able to demonstrate (e.g. based on retrospective data) site suitability in terms of recruitment, sufficient time, and adequate resources (both staff and facilities) to conduct the trial properly and safely, and to complete it within the agreed period.
The Principal Investigator and other delegated staff should ensure the required and sufficient amount of time for all scheduled monitoring visits; they should also agree and accept monitoring visits and audit/inspection requirements and procedures.

5.3 Contract Research Organization

The organization contracted to perform trial start-up related activities, monitoring, data management and statistical analysis, directly or through subcontractors, is OPIS S.r.l. Palazzo Aliprandi - Via Matteotti, 10 - 20832 Desio (MB) - Italy.
OPIS is also in charge of some activities related to SAE reporting and of supplying the electronic Case Report Form (eCRF), the e-diary and the module for treatment allocation.

5.4 Central Laboratory

Routine haematology and biochemistry, serology, and nasopharingeal swabs for SARS-COV-2 will be performed by the local laboratory of each participating centre.

Immunogenicity analyses will be performed by INMI Lazzaro Spallanzani - Via Portuense, 292, Roma.

6. SELECTION AND WITHDRAWAL OF SUBJECTS

Healthy males and non-pregnant females, 18 to 65 years of age inclusive, meeting all eligibility criteria will be enrolled.

A Male:Female 1:1 ratio (approximately) will be maintained within each cohort.

Screening can occur up to 30 days prior to the vaccination. Participant Inclusion and Exclusion Criteria must be confirmed by the Principal Investigator or appropriate sub-investigator.

No exemptions will be granted on Inclusion or Exclusion criteria.

Information regarding this trial may be provided to potential participants in different forms to promote the recruitment in the study, provided that the recruitment process and all materials were approved by the local EC prior to its use.

A suitable number of subjects will be screened, in order to enroll up to a total of 160 or 240 participants in the two study Phases, as follows:

Phase I

A total of 80 subjects, i.e. 20 in each of the 4 cohorts (Table 1):

Table 1. Phase I treatment group size, dose and dosing schedule

Cohort size (n)	Dose	Schedule
Cohort 1 (n=20)	0.5 mg	Prime-Boost (PB)
Cohort 2 (n=20)	1 mg	Prime-Boost (PB)
Cohort 3 (n=20)	2 mg	Prime-Boost (PB)
Cohort 4 (n=20)	2 mg	Prime (P)

Phase II

Additional subjects will be selected for enrolment in the expansion cohort(s) to reach a sample size of 100 subjects in each expanded dose schedule cohort. Therefore, if only one dose schedule is selected for expansion, a total of additional 80 subjects will be enrolled, while if two dose schedules are selected for expansion, a total of additional 160 subjects will be enrolled.

6.1 Inclusion criteria

Subjects meeting all the following inclusion criteria at screening will be eligible for enrolment in the study:

1. Signed and dated informed consent obtained before undergoing any study-specific procedure
2. Healthy male or female aged ≥18 and ≤ 65 years
3. Body Mass Index >18.5 and ≤30 kg/m^2
4. Vital signs within the following values or ranges:
   a. Body temperature ≤ 37,5 °C
   b. Pulse frequency ≥51 and ≤100 beats per minute

c. Diastolic BP ≥60 mmHg, ≤ 90 mmHg

d. Systolic BP ≥ 90 mmHg, ≤ 140 mmHg

e. Respiratory rate ≥ 12 breaths per minute, ≤ 16 breaths per minute

5. ECG at screening normal or with no clinically significant findings (pre-excitation syndromes, e.g., Wolff-Parkinson-White syndrome are absolute exclusion criteria)
6. Laboratory examinations within normal reference range or with no clinically significant abnormalities
7. Absence of any respiratory and flu-like symptoms
8. Non-pregnant women of childbearing potential, willing to practice a highly effective method of contraception from enrolment up to study completion or at least 90 days after the last vaccination in case of withdrawal
9. For sexually active men with a female partner of childbearing potential, willingness to use a condom and to refrain from donating sperm from enrolment up to study completion or at least 90 days after the last vaccination in case of withdrawal
10. Agreement to refrain from blood donation during the course of the study
11. Able and willing to comply with all study procedures.

6.2 Exclusion criteria

Subjects meeting any of the following criteria at screening are to be excluded from the study:

1. History of confirmed infection with SARS-CoV-2, by positive nasopharingeal swab or by positive serological test for SARS-CoV-2 antibodies
2. Positive serological test for SARS-CoV-2 antibodies at screening
3. Subjects at high risk of SARS-CoV-2 infection prior or during the trial, including:
   a. subjects with any known exposure in the 4 weeks before enrolment
   b. close contacts of suspected or confirmed COVID-19 or SARS-CoV-2 infection cases
   c. subjects quarantined for any reason
   d. frontline healthcare professionals working in Emergency departments, ICU and other higher risk healthcare areas
4. Positive serological tests for:
   a. Hepatitis B surface antigen (HBsAg)
   b. Hepatitis C antibodies
   c. Human Immunodeficiency Virus (HIV) antibodies
5. Subjects with any of the following specific contraindications, even in medical history:
   a. Type 2 diabetes or glucose intolerance, even if controlled

b. Hypertension, even if controlled
c. COPD
d. Any cardiac disease, even if not evident at ECG
e. Pacemaker

6. Use of any investigational drugs/treatments, or enrolment in a clinical trial during the 6 months preceding screening
7. Prior administration of any vaccine in the 2 weeks preceding screening
8. Administration of any monoclonal or polyclonal antibody product within 4 weeks preceding screening
9. Administration of any blood product within 3 months of screening
10. Current or prior administration, within the 6 months preceding screening, of immunosuppressants (inhaled, topical skin and/or eye drop-containing corticosteroids; a short course of corticosteroids, defined as ≤20 mg/day prednisone or equivalent for 10 days, and low-dose methotrexate are allowed until 4 weeks prior to screening)
11. Any prior major surgery or any chemio- or radiation therapy within 5 years of screening
12. Current or suspected immunosuppressive or immunodeficient state, including HIV infection, asplenia, recurrent severe infections
13. Active, known, or suspected autoimmune disease (except mild psoriasis, well-controlled autoimmune thyroid disease, vitiligo or stable coeliac disease not requiring immunosuppressive or immunomodulatory therapy)
14. Bleeding disorders (e.g. coagulopathy or platelet disorder or coagulation factor deficiency) or prior history of significant bleeding or bruising following IM injections or venipuncture
15. History of seizures or mental illness
16. History of allergy to vaccines or of severe allergic reaction of any kind
17. Metal implants within 20 cm of the planned site(s) of injection
18. Presence of keloid scar formation or hypertrophic scar, or other clinically significant medical condition at the planned site(s) of injection
19. Any abnormality or permanent body art (e.g. tattoos) that would interfere with the ability to observe local reactions at the injection site in the deltoid area
20. History of alcohol or drug abuse during the 12 months preceding the screening
21. Pregnancy (i.e. positive pregnancy test) or willingness/intention to become pregnant during the study
22. Breastfeeding

23. Any other clinically relevant disease and condition that, in the opinion of the Investigator, may jeopardize efficacy or safety assessments or may compromise the subject's safety during trial participation.

6.3 Criteria to be confirmed before each vaccination

The day of first (prime) vaccination, only subjects meeting all the following criteria will be eligible for vaccination:

1. Negative SARS-CoV-2 nasopharingeal swab performed in the previous 72 hours
2. No close contacts with suspected or confirmed COVID-19 or SARS-CoV-2 infection cases and no quarantine for any reason since screening
3. Absence of any respiratory and flu-like symptoms
4. Vital signs within the following values or ranges:
    a. Body temperature ≤ 37°C
    b. Pulse frequency ≥51 and ≤100 beats per minute
    c. Diastolic BP ≥60 mmHg, ≤ 90 mmHg
    d. Systolic BP ≥ 90 mmHg, ≤ 140 mmHg
    e. Respiratory rate ≥ 12 breaths per minute, ≤ 16 breaths per minute
5. Negative pregnancy test.

The day of second (boost) vaccination, only subjects meeting all the following criteria will be eligible for vaccination:

1. Negative SARS-CoV-2 nasopharingeal swab performed in the previous 72 hours
2. Absence of any respiratory and flu-like symptoms*
3. Vital signs within the following values or ranges:
    a. Body temperature ≤ 37°C
    b. Pulse frequency ≥51 and ≤100 beats per minute
    c. Diastolic BP ≥60 mmHg, ≤ 90 mmHg
    d. Systolic BP ≥ 90 mmHg, ≤ 140 mmHg
    e. Respiratory rate ≥ 12 breaths per minute, ≤ 16 breaths per minute
4. Negative pregnancy test.

*in case of fever, with negative SARS-CoV-2 nasopharingeal swab, the subject can be re-evaluated 1 week after, and vaccinated only in case of body temperature ≤ 37°C and availability of a second negative SARS-CoV-2 nasopharingeal swab.

6.4 Withdrawal of subjects

A participant will be considered to have completed the study when s/he completes all scheduled vaccine administrations, study procedures and follow-up visits.

In case of withdrawal from study participation, the following general rules apply:

- When a participant withdraws from the study, the reasons for withdrawal shall be recorded by the Investigator on the Medical File and on the relevant page of the eCRF.
- All participants prematurely discontinuing the trial must be seen no later than 30 days after study discontinuation.

Withdrawal from study participation may occur under the following circumstances:

- Withdrawal of consent

  Every participant is free to withdraw from the trial at any time upon request. However, although spontaneous unilateral withdrawal of participants from the study is theoretically and ethically acceptable, the subjects would be requested to explain to the Investigator the reasons for such decision.

- Protocol deviation

  Subjects with major protocol deviations will be evaluated by the Sponsor in order to decide about participant discontinuation.

- Occurrence of adverse event (AE)/serious adverse event (SAE)

  In case of withdrawal due to AEs/SAEs, the Investigator will follow up the participants until resolution or acceptable stabilization of the event and document all the relevant information as applicable.

- Pregnancy

  In case of pregnancy, the Investigator will follow up the participant, or the partner of the male trial participant, until the final outcome is known, and will document all the relevant information, as applicable. Pregnancy resulting in an abnormal outcome (e.g. congenital anomaly) should be monitored to follow the newborn development for an appropriate post-delivery period.

- Loss to follow-up

  Every effort should be made to contact the participants in order to perform a final visit. Participants who fail to return for a final visit will be reached by telephone (two phone calls, at least) or requested in writing to return for a visit (a copy of the letter/e-mail must be kept by the Investigator, together with the source documentation) in an attempt to have them comply with the protocol.

- At the discretion of the Investigator

  Any other condition that, in the opinion of the Investigator, may jeopardize the study conduct according to the protocol, or when the Investigator feels that it is in best interest of the participant to discontinue.

The Investigator will also withdraw all participants from the study if the trial is terminated, provided that this has been anticipated in the information given to participants.

6.4.1 *Replacement*

Subjects will be replaced in the following circumstances:

- Subjects belonging to the PB groups, who refuse to undergo the boost administration, or discontinue the study before the boost administration
- Subjects not matching the criteria for vaccination the day of boost administration.

7. TREATMENTS, LOGISTICS, AND MATERIALS

7.1 Identity of the investigational product

The identity of the investigational product COVID-*e*Vax is as follows:

- Name: COVID-*e*Vax
- Description: A clear, colourless, aqueous solution for injection (sterile, endotoxin free) free from visible particulates
- Size: 5635 base pair
- pH: 7.4 ± 0.5
- Strength/concentration: 4 mg/mL (vials at the nominal fill volume of 1 mL)
- Dosage regimen: 0.5 mg, 1 mg or 2 mg for each administration
- A complete record of batch numbers of all investigational products will be maintained throughout the study
- Use-by date: as reported on the labels

7.2 Subject identification and allocation of treatments

At screening, each subject will be identified with a unique identification number (ID) consisting of 4 digits: 1 digit for the centre (site number), and 3 progressive digits for the participant.

The ID is centrally assigned to each participant; in order to obtain the ID, the Investigator should access the eCRF and create a new participant.

After screening visit is completed, only those participants fulfilling all the inclusion criteria and none of the exclusion criteria, and meeting the criteria foreseen to be evaluated on D1 (see section 6.3) can be assigned the study drug and can start the treatment.

A specific module integrated in the eCRF will assign to each participant the numbered vial/s of vaccine, selected from the stocks available at the site.

The assignment of the vaccine vial will be done on D1 for participants assigned to the Prime group and on D1 and D29 for participants assigned to Prime-Boost groups.

An e-mail will be sent to the site and to the Rottapharm Biotech to notify the vial numbers assigned.

7.3 Packaging and labelling

COVID-*e*Vax manufacturing, primary packaging and QC testing, stability, secondary packaging, labelling and final QP release for administration are performed in accordance with GMP and GCP.

The manufacture and release of COVID-*e*Vax drug substance, QC testing and stability studies of COVID-*e*Vax drug substance and drug product are performed at the following facility:

Biomay AG
Lazarettgasse 19, 1090 Vienna - Austria

The manufacture, primary packaging and vials labelling of COVID-*e*Vax drug product are performed at the following facility:

Polymun Scientific Immunbiologische Forschung GmbH
Donaustrasse 99 - 3400 Klosterneuburg - Austria

Secondary packaging, labelling of the drug product (carton boxes) and final QP release for administration are performed at:

Biomay AG
Lazarettgasse 19, 1090 Vienna - Austria

COVID-*e*Vax will be supplied to sites packaged in packs containing 8 numbered vials each. The vials will be identified with a three-digit number, from 001 to 999 and labelled in local language, with at least the following information:

- Protocol/Study No.
- Batch/Packaging No.
- Vial No.
- Pharmaceutical form, dosage, and route of administration
- Sponsor's name
- Storage instructions
- Directions for use

The packs will be labelled in local language, with at least the following information:

- Protocol/Study No.
- Batch/Packaging No.
- Vial No.
- Pharmaceutical form, dosage, and route of administration
- Sponsor's name
- Partner, name, address, and telephone number
- Storage instructions
- Use-by date
- Directions for use
- "For clinical trial use only"

Sample labels will be filed in the Trial Master File (TMF).

7.4 Supply, storage, accountability and return

Rottapharm Biotech will provide the sites with COVID-*e*Vax, together with the relevant documentation.

A temperature -20±5°C should be maintained both for transport and for storage.

COVID-*e*Vax supplied to the centres must be stored carefully, safely, and separately from other drugs.

The shipments will be performed by an authorized courier that will ensure continuous monitoring of the shipment temperature. Records of temperature measures, throughout the whole shipment period, will be provided by the courier and filed in the appropriate TMF section.

After reception at the centre, the Site/Pharmacy staff must complete and return to Rottapharm Biotech the Shipping/Receipt Form, verifying the receipt and integrity of the product.

Responsibility for Investigational Products accountability at the trial site will be of Principal Investigator; assignment of relevant duties to a Pharmacist or other adequately authorized delegate may be required/allowed, under the supervision of Principal Investigator. The Principal Investigator should maintain records of delivery, inventory at the site, use by each participant, and return or alternative disposition for unused products.

Investigational product inventory and dispensing will be documented as follows:

- An Inventory Form, documenting the amounts received, administered or unused, and returned will be maintained and updated.
- An Accountability/Administration Form, documenting the vaccine administration/s for each participant will be maintained and updated.

The Principal Investigator should ensure safe and secure IMP storage according to specifications provided by the Rottapharm Biotech, and that the IMP will be used strictly in accordance with the study protocol.

Appropriate documentation of adequate temperature storage at pharmacy/site should be kept in the ISF.

In case the pharmacy/site has an internal system of temperature recording, the Investigator should provide a copy of this documentation, otherwise a Temperature Log provided by Rottapharm Biotech should be filled in.

A numbering system, in accordance with all requirements of GMP, will be used. This will ensure that any study medication can be identified and traced back to the original bulk ware/batch of the active ingredients.

7.5 Electroporation Medical Device

Each vaccination consists of a COVID-*e*Vax injection, followed by a sequence of electrical pulses delivered intramuscularly via the IGEA proprietary electroporator device available in the EU market, the Cliniporator®. In this section a brief description of Cliniporator® (the electrical pulse generator) and of the new ElectroPoration System (EPS) Gun (the electrodes) developed by IGEA and used in the study is provided.

The site personnel using the device will be adequately trained for its use.

7.5.1 *Electrical pulse generator (Cliniporator®)*

The electrical pulse generator Cliniporator® is the most advanced device for tissues EP (Figure 4).

Cliniporator® enables the application of EP applying high and low voltage electric pulses that allow the intracellular transfer of molecules not or little permeant the cell membrane. During the EP procedure, the Cliniporator® measures the voltage and current wave forms applied and displays them in real time. Cliniporator® has a safety control system that blocks the pulse output if the current exceeds 5 Amps. The treatment data are stored inside an archive.

Cliniporator® complies with electrical safety standards EN 60601-1, complies with the requirements of the European directives for medical devices 93/42/EEC and 2007/47/EEC and it is marked CE0051 under the control of the Notification Body IMQ.

- Registration to Italian Medical Device Repertory Number:295232
- EN 60601-1 Classification
- Protection against electrical risks: Class I
- Protection against electric shock: BF

Figure 4. Cliniporator® Medical Device (electrical pulse generator)

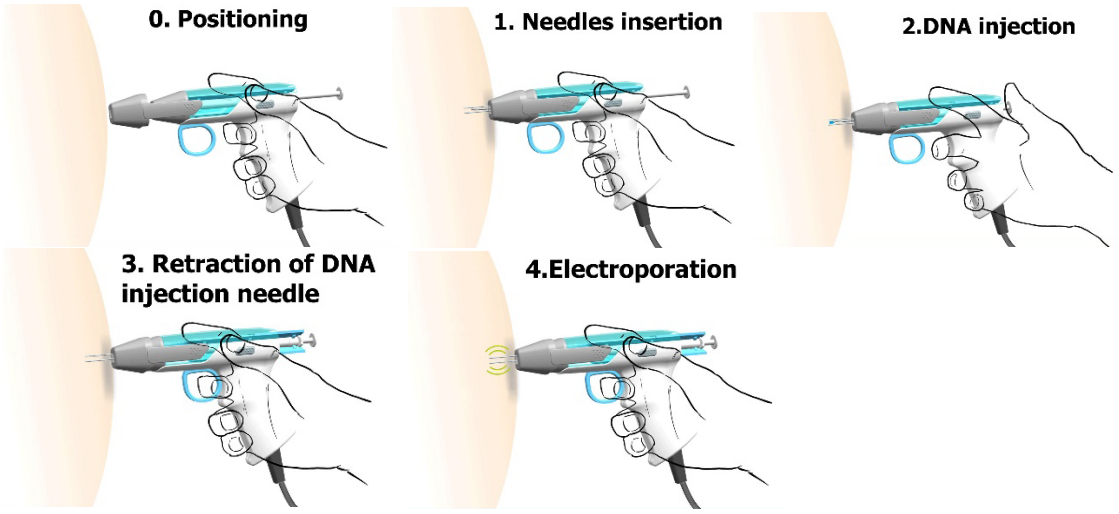
7.5.2 *The electrodes (EPSGun)*

The electroporation technology has been optimized by IGEA developing a new dedicated EPSGun (CE mark medical device, certificate number 1673/MDD dated 06 November 2020), (Figure 5).

Figure 5. IGEA EPSGun

The whole DNA vaccine delivery procedure through the EPSGun is an intuitive and handful procedure that combines the injection of the DNA vaccine and the delivery of the electrical pulses and it is completed in few seconds (Figure 6).

Figure 6. Procedure for DNA Injection and Delivery of the Electrical Pulses



Briefly, the EPSGun accommodates the syringe containing the DNA vaccine, within one single motion. The EPSGun allows the insertion of both the electrode needles and the vaccine needle into the muscle (1). By pressing the syringe plunger, the DNA vaccine will be injected (2), by pulling the trigger the syringe needle will be retracted (3), and finally the electrical pulses will be delivered by pressing a pedal connected to the pulse generator (4).
The EPSGun guarantees high reproducibility and reliability of vaccine delivery, regardless of the manual skills of the operator.
The EP needles (4 needles, diameter 0.45 mm) are hidden under a sliding cap integrated into the EPSGun, to reduce perceived pain potentially associated to anxiety or fear.

7.6 Vaccine administration

The vaccination/EP procedure will be performed by qualified personnel. Any licensed healthcare provider designated to perform the procedure must have the appropriate license to administer vaccinations or parenteral drugs to participants.

All individuals designated to perform the EP procedure must receive an adequate and specific training on the device regardless of their medical qualification.

In study Phase I, 4 cohorts are planned, as follows:

- 0.5 mg PB (Prime-Boost, 4 weeks apart) - Total dose: 1 mg
- 1 mg PB (Prime-Boost, 4 weeks apart) - Total dose: 2 mg
- 2 mg PB (Prime-Boost, 4 weeks apart) - Total dose: 4 mg
- 2 mg P (Prime) - Total dose: 2 mg

Each COVID-*e*Vax administration will consist of an IM injection of 0.5 mL (for all doses) into the deltoid muscle of the right arm, followed immediately by EP. For participants belonging to PB groups, the second administration will be performed in the same arm.

The 2 mg dose is obtained by picking up 0.5 mL from the content of the 4 mg/mL vial with a 1 mL syringe (with a 22G-38mm (1 ½") needle) and injected.

The lower doses are obtained diluting the content of the vial with isotonic saline solution (0.9% w/v), immediately before the administration.

The 1 mg dose is obtained diluting 0.5 mL of the 4 mg/mL formulation with 0.5 mL of saline in a sterile sealed empty vial; then 0.5 mL of the dilution is taken with a 1 mL syringe (with a 22G-38mm (1 ½") needle) and injected.

The 0.5 mg dose is obtained diluting 0.5 mL of the 4 mg/mL formulation with 1.5 mL of saline in a sterile sealed empty vial; then 0.5 mL of the dilution is taken with a 1 mL syringe (with a 22G-38mm (1 ½") needle) and injected.

The syringe is to be inserted in the EPSGun; then, by pressing the syringe plunger, the DNA vaccine will be injected. By pulling the trigger the syringe needle will be retracted and finally the electrical pulses will be delivered by pressing a pedal connected to the pulse generator.

After IM administration and EP, the injection site will be covered with a sterile dressing.

Participants will be instructed to use analgesics (e.g. paracetamol) in case of pain.

The following EP parameters will be used during the study:

- Number of pulses per treatment = 4
- Voltage amplitude= 40V (corresponding to an electric field strength of 100 V/cm)
- EP pulse duration = 5 milliseconds/pulse
- Interval separating pulses = 5 milliseconds
- IM injection depth = 16 mm
- EP injection depth = 21 mm

Participants will stay at site for observation for 4 hours after the vaccination. The sterile dressing will be removed 30±5 minutes after vaccination and the injection site will be inspected every 30±5 minutes, until the volunteer leaves the site.
A thermometer, a ruler and a diary will be given to each participant, with instructions on use, along with the 24-hour emergency telephone number, to make him/her able to contact the study physician if needed.

7.7 Blinding

Blinding is not applicable, this is an open-label study.

7.8 Prior and concomitant medications and treatments

7.8.1 *Excluded medications and treatments*

Participants must have not received any investigational drugs/treatments (or enrolled in a clinical trial) during the 6 months preceding screening, or administration is planned at any time during the study period.

Participants must not have been vaccinated (any vaccine) during the 2 weeks preceding the screening and must not have received polyclonal or monoclonal antibodies during the 4 weeks preceding screening; administration of vaccines or polyclonal or monoclonal antibodies should not be planned at any time during the study period; the only exception is flu-vaccine, which can be administered at least 4 weeks after the last COVID-*e*Vax administration.

Administration of immunosuppressants is not allowed during the study and within the 6 months preceding the screening. Inhaled, topical skin and/or eye drop-containing corticosteroids, a short course of corticosteroids (defined as ≤20 mg/day prednisone or equivalent for 10 days) and low-dose methotrexate only, are allowed until 4 weeks prior to screening.

Any medications that in the opinion of the Investigator might interfere with the evaluation of COVID-*e*Vax should not be used by the participants during the study-reporting period unless clinically indicated as part of the participant's health care.

7.8.2 *Permitted medications and treatments*

Analgesic and anti-inflammatory medications for symptoms relief after the vaccine administration and any treatment that the Investigator considers strictly necessary for the participants's welfare are permitted during the study, no other medications are permitted.

All concomitant medications will have to be recorded in the eCRF (both prescription and over-the-counter medications). If changes occur during the study period, documentation of drug dosage, frequency, route, and date will also be reported in the eCRF.

All medications that the participant is taking during the 28 days before first dose of trial treatment administration must be recorded in the Prior and Concomitant Medication Form. In addition, any change in concomitant medications or new medications added during the study must be recorded in the Prior and Concomitant Medication Form.

7.9 Other study supplies

7.9.1 *eCRF*

Rottapharm Biotech shall also provide the Investigators with a suitable eCRF and will give adequate training about its usage.

The eCRF is divided into sections for the different phases of the study.

Further, specific sections for Prior and Concomitant Medications, AEs and Study Outcome have to be completed for all participants, including screening failures/withdrawals/drop-outs. The language to be used is English.

7.9.2 *Participant Diary*

Study participants will be provided with an e-diary and asked to record the occurrence of solicited local and systemic AEs (as detailed in Section 11.1.5) and medications use because of the AEs (i.e. antipyretic, pain medications) for 7 days following each vaccine administration.

The Investigator will periodically review the Participant Diary. At the clinic visits, the Investigator will collect and record on the eCRF additional information about the

medications used (e.g. brand name, indication, dosage form, dose unit, dose frequency, daily dose, route of administration, start and stop dates, pharmaceutical formulation), if any.

8. EVALUATION CRITERIA

The assessments hereinafter listed are applicable to both Phase I and Phase II.

8.1 General assessments

- Informed Consent Forms and Screening Log
- Demographics, Baseline Disease Characteristics and Medical History

8.2 Safety assessments

- Solicited local and systemic Adverse Events
- Unsolicited Adverse Events
- Laboratory examinations
- Physical examination
- Vital signs

8.3 Immunogenicity assessments

- Quantitative binding antibodies anti-S and anti-N SARS-CoV-2 proteins
- SARS-CoV-2 neutralizing antibody titer
- Antigen-specific cellular immune response.

9. EVALUATION METHODS

9.1 General assessments

9.1.1 *Informed Consent Forms and Screening Log*

Participants may be recruited by use of an advertisement. In this case the advertisement will be submitted to the EC for approval.

Written informed consent for participation in the study must be obtained before performing any study-related procedures. Informed Consent Forms for enrolled subjects and for subjects who are not subsequently enrolled will be maintained at the study site.

Subjects will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

- ✓ Participation in the study is entirely voluntary
- ✓ Refusal to participate involves no penalty or loss of medical benefits
- ✓ The volunteer may withdraw from the study at any time
- ✓ The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- ✓ The study involves research of an investigational vaccine
- ✓ There is no direct benefit from participating
- ✓ Participation in the study exposes to potential risks (including the risk of ADE)
- ✓ The volunteer's GP could be contacted to corroborate their medical history
- ✓ The volunteer's blood samples taken as part of the study will be stored indefinitely and samples may be sent outside Italy and Europe to laboratories in collaboration with the Sponsor. These will be anonymised.

The aims of the study and all tests to be carried out will be explained. The volunteers will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the Investigator Site File. These forms will also be signed and dated by the Investigator.

All screening evaluations must be completed and reviewed by the Investigator to confirm that participants meet all eligibility criteria before inclusion. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
An Identification number (ID) will be centrally assigned to each participant; in order to obtain the ID, the Investigator shall access the eCRF and create a new participant.

9.1.2 *Demographics and Medical History*

Demographic data will include age, sex, and self-reported race/ethnicity.
Medical history includes clinically significant diseases, prior surgeries, reproductive status, smoking habits and use of alcohol, and drugs of abuse. In addition, all medications (e.g., prescription drugs, over-the-counter drugs) used by the participant during the 28 days prior to initiation of the study drugs will be recorded.

9.2 Safety assessments

9.2.1 *Recording of adverse events*

During the whole study, particular attention will be paid to the occurrence of any AE, either observed by the Investigator/nursing staff or reported by the participants.

Study participants will be directly observed at the clinical site by study personnel for a minimum of 4 hours after each vaccination and afterwards contacted by telephone by study personnel in the evening of the day of vaccination and 24 hours after vaccination, to be enquired for immediate reactions and to decide if an unscheduled visit is deemed necessary.

Before leaving the site, after a minimum of 4 hours, the participants will be trained on how to use the thermometer, the ruler and the Participant Diary.
They will be asked to take and record their temperature daily (in the evening) and to note local and systemic solicited AEs (listed Section 11.1.5) they might experience, during the first 7 day period after each vaccine administration.

In addition, at each clinic visit, study participants will be queried regarding the occurrence of any unsolicited AEs since the last visit.

Instruction on AE collection, recording, assessment, and reporting are given in Section 11.

9.2.2 *Laboratory tests*

Laboratory examinations for routine safety evaluations, both for Phase I and Phase II, will be performed at the local laboratory.

- HEMATOLOGY: white blood cell (WBC) count and differential, red blood cell (RBC) count, haemoglobin, haematocrit, platelet count
- CHEMISTRY: glucose, blood urea nitrogen (BUN) or urea, creatinine, sodium (Na), potassium (K), total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), creatine phosphokinase (CPK), total proteins, c-reactive protein (CRP)
- URINALYSIS: pH, ketones, proteins, glucose, haemoglobin, leucocytes, nitrites

  Hematology, Chemistry and Urinalysis are to be performed:

  - at screening
  - on D1
  - on D3, during Phase I only
  - at Week 1, 4, 5 (for subjects belonging to PB groups only), 8, 12 and 24
- SEROLOGY
  - HIV testing
  - HBsAg, antibodies against HBsAg, total HBcAg antibody (anti-HBcAb) - (*HBV DNA should be obtained if the participant has a negative serology for HBsAg and a positive serology for anti-HBcAb)*
  - HCV antibody (anti-HCV) – (*HCV RNA should be obtained if the participant tests positive for anti-HCV)*

To be performed:

- At screening

- SARS-CoV-2 nasopharyngeal swab for molecular testing (RT-PCR)

  To be performed:

  - 72 hours before vaccination on D1 and before vaccination on D29 (for subjects belonging to PB groups only): results should be available before vaccination(s)

- SARS-CoV-2 quantitative serological testing (IgG)

  To be performed:

  - at screening

PREGNANCY TEST: serum/urine β-HCG (human chorionic gonadotropin)

To be performed in all women of childbearing potential:

- at screening
- before vaccination on D1 and before vaccination on D29 (for subjects belonging to PB groups only)
- at Week 4, 8, 12 and 24

All laboratory evaluations, but serology and SARS-COV-2 serology, should be performed also in case of premature study termination.

The volumes of blood samples to be drawn from each participant during the entire duration of the study are displayed in Table 4 (for Phase I) and Table 5 (for Phase II).

9.2.3 *Physical examination*

A complete physical examination, including an evaluation of the head, eyes, ears, nose, and throat and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems, will be carried out at screening and at the last study visit.

Targeted physical assessments (including injection site evaluation) will be performed at each following visit as determined by the Investigator or directed per participant complaints.

Clinically significant findings of the physical examinations at screening will be documented as concomitant diseases in the medical history section of the eCRF.

Changes from baseline abnormalities should be recorded in participant notes. New or worsened clinically significant abnormalities should be recorded as adverse events in the Adverse Event section of the eCRF.

9.2.4 *Vital signs and ECG*

Vital signs will include measurements of pulse rate, respiratory rate, systolic and diastolic blood pressures while the participant is in a seated position (after ≥10 minutes at rest), and

body temperature. They should be determined and recorded at screening and at every subsequent visit.

Clinically significant abnormalities in vital signs will be graded for AE using the FDA toxicity grading scales for healthy volunteers enrolled in vaccine clinical trials[38] (see Appendix I). A 12-lead ECG is required at screening for all participants to determine eligibility, and 1 hour after each vaccine administration.

The ECG should include measurements of ventricular rate, PR, QRS, QT, QTc with assessment as to whether the ECG is normal or abnormal. Abnormal ECGs will be interpreted as clinically significant or not clinically significant. Participants will be excluded in the event of a clinically significant abnormal ECG at baseline.

Paper or electronic copies of ECG tracings will be kept as part of the participant's study file at the site. ECG abnormalities must be documented in the eCRF.

9.3 Immunogenicity assessments

The immunogenicity assessments will involve quantitative assays aimed to measure both humoral and cell-mediated immunity.

The preparation of blood samples and shipping instructions for humoral and cellular immunogenicity assays will be outlined in a specific manual prepared by the Central Laboratory.

9.3.1 *Humoral Immunogenicity Assays*

The following humoral immunogenicity assays will be performed on serum collected at screening and at selected time points after vaccine administration (for details on the timing and the volumes of venous blood to be withdrawn in Phase I and Phase II, please refer to Section 10, Table 4 and Table 5, respectively):

- Quantitative binding antibodies anti-S and anti-N SARS-CoV-2 proteins
- SARS-CoV-2 neutralizing antibody titer.

Binding antibodies (S and N proteins)

Quantification of antibody response to RBD will be conducted by conventional Enzyme-Linked Immunosorbent Assay (ELISA) assays. Blood samples will be collected according to the procedures indicated by the immunoassay kits manufacturer or by the specific assay procedure developed by the Central Laboratory. Serum will be cryopreserved at -80 °C in an adequate number of aliquots for each time point for each determination until analysis.

SARS-CoV-2 neutralizing antibody titer

Quantification of neutralizing antibody titers will be performed by the Central Laboratory using a SARS-CoV-2 neutralization assay on Vero cells.

9.3.2 *Cellular Immunogenicity Assays*

The following cellular immunogenicity assays will be performed on peripheral blood mononuclear cells (PBMCs) collected at screening and at selected time points after vaccine

administration (for details on the timing and the volumes of venous blood to be withdrawn in Phase I and Phase II, please refer to Section 10, Table 4 and Table 5):

T cell responses to SARS-CoV-2 Spike protein or RBD protein will be assessed by the IFN-γ ELISpot.

In addition, the intracellular cytokine staining (ICS) assay will be used as exploratory assessment to characterize the CD4 and CD8 responses to SARS-CoV-2 Spike protein or RBD protein on PBMCs (for Th1: IFNγ, IL-2 and TNFα; for Th2: IL-4, IL-5 and IL-13; for Th17: IL-17A).

9.4 Time schedule of the Immunogenicity Assays

The sera for the humoral responses (binding and neutralizing antibody titers) and the PBMCs for determination of the cellular responses will be collected at different time points as shown in Table 4 and Table 5.

Samples should be frozen, stored and shipped at -80°C.

The principal analyses for humoral and cellular responses will be performed on samples at selected clinic visits, while samples collected at the remaining visits will be analysed at a later stage and only if deemed necessary (e.g. for the dose/schedule(s) selected for Phase II expansion, or during this selection process, or for any reason requiring better assessment of the kinetics of the immunological response).

10. SCHEDULE OF EVALUATIONS

The study flowcharts applicable for Phase I and Phase II of the study are given below in Table 2 and Table 3, respectively, while the approximate volumes of the venous blood to be collected for routine clinical laboratory and immunogenicity evaluations are shown in Table 4 and Table 5.

Table 2. Study flow chart for Phase I

Procedure	Screen. -30/-1	Day 1	Day 3	Wk1 (D8 ±1)	Wk2 (D15 ±1)	Wk4 (D29 ±1)	#Wk5 (D8±1 post boost)	Wk6 (D43 ±2)	Wk8 (D57 ±2)	Wk12 (D85 ±3)	Wk16 (D113 ±3)	Wk24 (D169 ±5)
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Informed Consent	X											
Medical history	X	X										
Demographics	X											
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[1]	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X[2]	X	X	X	X[2]	X	X	X	X	X	X
12-lead ECG	X	X[3]				X[3]						
Hematology	X	X	X	X		X	X		X	X		X
Chemistry[4]	X	X	X	X		X	X		X	X		X
Urinalysis	X	X	X	X		X	X		X	X		X
Serology[5]	X											
Nasopharingeal swab for SARS-CoV-2		X[6]				X[7]						
SARS-COV-2 serology (quantitative)	X											
Pregnancy test[8]	X	X[9]				X[9]			X	X		X
Binding antibodies[10]		X		X	X	X	X	X	X	X	X	X
Neutralizing antibodies[10]		X				X			X			X
Cellular immune response[10]		X			X	X			X	X		X
Vaccination		X[11]				X[12]						
Adverse Events	←----------											----------→
Participant Diary		X[13]	X[14]	X[15]		X[13]	X[15]					
Telephone contact		X[16]				X[16]						

#Visit not applicable to subjects belonging to prime group

[1] Full physical examination at screening and last study visit only; targeted examination at other visits, as determined by Investigator or per participant complaints

[2] Vital signs to be performed pre- and 30-min-post- vaccination in subjects undergoing vaccine administrations, at any time for the others

[3] One (1) hour after vaccine administration

[4] Sodium (Na), potassium (K), glucose, BUN or urea, Cr, total bilirubin, ALT, AST, CPK, total proteins, CRP

[5] HIV antibodies, HBsAg, HCV antibodies

[6] Nasopharingeal swab to be performed in the 72 hours before vaccination on D1

[7] Nasopharingeal swab to be performed in the 72 hours before vaccination on D29 for PB groups only

[8] Serum/Urine pregnancy test
[9] On D1 before vaccine administration for all subjects; on D29 before vaccine administration for PB groups, at any time for the others
[10] Details on volumes required for the immunogenicity assessments are provided in Table 4
[11] Vaccine administration for all participants
[12] Vaccine administration for participants belonging to PB groups only
[13] Diary hand-over and training on use to participants
[14] Diary check
[15] Diary collection
[16] In the evening of the day of vaccination and 24 hours after vaccination

Table 3. Study flow chart for Phase II

Procedure	**Screen. -30/-1**	**Day 1**	**Wk1 (D8 ±1)**	**Wk2 (D15 ±1)**	**Wk4 (D29 ±1)**	**#Wk5 (D8±1 post boost)**	**Wk6 (D43 ±2)**	**Wk8 (D57 ±2)**	**Wk12 (D85 ±3)**	**Wk24 (D169 ±5)**
	V1	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**
Informed Consent	X									
Medical history	X	X								
Demographics	X									
Concomitant Medications	X	X	X	X	X	X	X	X	X	X
Physical Exam[1]	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X[2]	X	X	X[2]	X	X	X	X	X
12-lead ECG	X	X[3]			X[3]					
Hematology	X	X	X		X	X		X	X	X
Chemistry[4]	X	X	X		X	X		X	X	X
Urinalysis	X	X	X		X	X		X	X	X
Serology[5]	X									
Nasopharingeal swab for SARS-CoV-2		X[6]			X[7]					
SARS-COV-2 serology (quantitative)	X									
Pregnancy test[8]	X	X[9]			X[9]			X	X	X
Binding antibodies[10]		X		X	X			X	X	X
Neutralizing antibodies[10]		X			X			X		X
Cellular immune response[10]		X		X	X			X	X	X
Vaccination		X[11]			X[12]					
Adverse Events	←----------									----------→
Participant Diary		X[13]	X[14]		X[13]	X[14]				
Telephone contact		X[15]			X[15]					

#Visit not applicable to subjects belonging to prime group

[1] Full physical examination at screening and last study visit only; targeted examination at other visits, as determined by Investigator or per participant complaints

[2] Vital signs to be performed pre- and 30-min-post- vaccination in subjects undergoing vaccine administrations, at any time for the others

[3] One (1) hour after vaccine administration

[4] Sodium (Na), potassium (K), glucose, BUN or urea, Cr, total bilirubin, ALT, AST, CPK, total proteins, CRP

[5] HIV antibodies, HBsAg, HCV antibodies

[6] Nasopharingeal swab to be performed in the 72 hours before vaccination on D1

[7] Nasopharingeal swab to be performed in the 72 hours before vaccination on D29 for PB groups only

[8] Serum/Urine pregnancy test

[9] On D1 before vaccine administration for all subjects; on D29 before vaccine administration for PB groups, at any time for the others

[10] Details on timing and volumes required for the immunogenicity assessments are provided in Table 5

[11] Vaccine administration for all participants

[12] Vaccine administration for participants belonging to PB groups only

[13] Diary hand-over and training on use to participants

[14] Diary collection

[15] In the evening of the day of vaccination and 24 hours after vaccination

Table 4. Phase I - Overview of by visit and cumulative blood sampling (mL)

Procedure		**Screen -30/-1**	**Day 1**	**Day 3**	**Wk1 (D8 ±1)**	**Wk2 (D15 ±1)**	**Wk4 (Day 29±1)**	**#Wk5 (D8±1 post boost)**	**Wk6 (D43 ±2)**	**Wk8 (D57 ±2)**	**Wk12 (D85 ±3)**	**Wk16 (D113 ±3)**	**Wk24 (D169 ±5)**
Visit No		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**
Vaccination			X[1]				X[2]						
Clinical Laboratory Evaluations		30	12[3]	12	12		12	12		12	12		12
Immunogenicity	Binding antibody titer		16[3]		(16)	16	16[3]	(16)	(16)	16	16	(16)	16
	Neutralizing antibody titer		16[3]				16[3]			16			16
	Cellular immune responses		40[3]			40	40[3]			40	40		40
Per visit Blood Volume total (mL)		30	84	12	28	56	84	28	16	84	68	16	84
Cumulative Blood Volume (mL)		30	114	126	154	210	294	294-#322	310-#338	394-#422	462-#490	478-#506	562-#590

#Visit not applicable to subjects belonging to prime group

Blood sampling in parentheses correspond to samples that will be analysed only if necessary, as described above in Section 9.4.

[1] Vaccine administration for all subjects

[2] Vaccine administration to subjects belonging to prime-boost groups only

[3] Samples to be collected before vaccine administration

Table 5. Phase II - Overview of by visit and cumulative blood sampling (mL)

Procedure		**Screen -30/-1**	**Day 1**	**Wk1 (D8 ±1)**	**Wk2 (D15 ±1)**	**Wk4 (Day 29±1)**	**#Wk5 (D8±1 post boost)**	**Wk6 (D43 ±2)**	**Wk8 (D57 ±2)**	**Wk12 (D85 ±3)**	**Wk24 (D169 ±5)**
Visit No		**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**
Vaccination			X[1]			X[2]					
Clinical Laboratory Evaluations		30	12[3]	12		12	12		12	12	12
Immunogenicity	Binding antibody titer		16[3]		16	16[3]			16	16	16
	Neutralizing antibody titer		16[3]			16[3]			16		16
	Cellular immune responses		40[3]		40	40[3]			40	40	40
Per visit Blood Volume total (mL)		30	84	12	56	84	12		84	68	84
Cumulative Blood Volume (mL)		30	114	126	182	266	266-#278	266-#278	350-#362	418-#430	502-#514

#Visit not applicable to subjects belonging to prime group

[1] Vaccine administration for all subjects

[2] Vaccine administration to subjects belonging to prime-boost groups only

[3] Samples to be collected before vaccine administration

11. ADVERSE EVENTS

AE definition, recording, and reporting follow the applicable procedures of Rottapharm Biotech and are described in detail in this section.

11.1 Definitions

11.1.1 *Adverse event*

An **adverse event (AE)** is defined as "any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment".

This definition includes the following events:

- intercurrent illnesses or injuries, changes in physical signs, new symptoms;
- laboratory or instrumental abnormalities considered to be clinically significant in the medical and scientific judgment of the Investigator;
- exacerbation or worsening of pre-existing conditions, including an increase in frequency and/or severity;
- conditions diagnosed or manifested after exposure to the IMP and/or any study medication, even though possibly present before the trial started;
- adverse events that are associated with study procedures, i.e. protocol-mandated interventions including those occurring prior to administration of study drug.

Symptoms, laboratory or instrumental abnormalities of a pre-existing condition which are present prior to clinical trial entry and do not worsen during the study will not be documented as AEs but will be documented as medical history (or other as applicable).

Surgical interventions planned before subject entered the study will not be documented as AEs.

Diagnostic and therapeutic procedures, of whatever nature, such as surgery (e.g. endoscopy, appendectomy) should not be reported as AE; instead, the medical condition for which this procedure was performed will be reported as AE (e.g. appendicitis), if the definition is met.

Abnormal laboratory findings and other abnormal investigational findings (e.g., on an ECG trace) should not be reported as AEs unless they are associated with clinical signs or symptoms or are considered otherwise medically important by the Investigator. If an abnormality fulfils these criteria, the identified medical condition (e.g., anemia, increased ALT) must be reported as the AE rather the abnormal value itself.

In this study, AEs can be further divided into solicited AEs and unsolicited AEs. Solicited AEs (foreseeable for a vaccine) are those for which the subjects will be specifically queried about their occurrence in the 7-day period after vaccination (see Section 11.1.5). Unsolicited AEs are those events that the subject reports without being queried about the specific event. Unsolicited AEs are AE other than the solicited AEs occurring within the first 7 days, and any AEs occurring after the first 7 days after vaccination.

11.1.2 *Serious adverse event*

A **serious adverse event (SAE)** is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event. Important medical events are events that may not be immediately life-threatening or result in death or hospitalization but may jeopardise the subject or may require an intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Medical and scientific judgement should be exercised in deciding whether an event is 'serious' in accordance with these criteria.

The primary cause of death or the event leading to death should be recorded and reported as an SAE. "Fatal" will be recorded as the outcome of the event. Death will not be recorded as separate event. Only if no cause of death can be reported (e.g., sudden death, unexplained death), might the death per se be reported as an SAE.

The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

An event has to be considered serious as per the hospitalization criterion only if the subject remains admitted overnight, including hospitalization for precautionary overnight observation after a medical occurrence.

The term disability is defined as a substantial disruption of a person's ability to conduct normal life functions, either reported or defined as per medical judgment.

Suspected transmission of an infectious agent via a study drug is to be considered a serious adverse reaction as per the important medical event criterion. This term applies only when a contamination of study treatment is suspected.

Seriousness should not be confused with severity (intensity) (see Section 11.2.3).

11.1.3 *Adverse reaction*

An **adverse reaction (AR)** is defined as "all untoward and unintended responses to a medicinal product related to any dose administered" (i.e. any adverse event judged by either the reporting Investigator or Rottapharm Biotech as having a reasonable causal relationship with the vaccination).

The definition covers also medication errors and uses outside what is foreseen in the present protocol, including misuse and abuse of the products.

The expression "reasonable causal relationship" means to convey in general that there is evidence or argument to suggest a causal relationship (see also Section 11.2.4).

11.1.4 *Unexpected adverse reaction*

An **unexpected adverse reaction** is an adverse reaction, the nature or severity of which is not consistent with the applicable medicinal product information, e.g. IB for an unauthorized IMP. Reports which add significant information on the specificity, increase of occurrence, or severity of a known already documented serious adverse reaction constitute unexpected events.

The reference document used for expectedness assessment in this study is detailed in Section 11.6.

11.1.5 *Solicited AE*

Solicited local and systemic AEs occurring during a 7-day follow-up period after each vaccination (i.e. the day of vaccination and the 6 subsequent days) will be reported in a diary. The subjects will be required to record the presence or absence of predefined AEs and the severity according to the tables provided in Section 11.2.3.

At the 7-day follow-up visit after each vaccination, the Investigator should verify each diary entry. Solicited AE collected in the diary should not be entered by the Investigator in the AE page of the eCRF, unless the solicited AE meets any of the following criteria:

- Solicited local or systemic AE leading to subject withdrawal from the study.
- Solicited local or systemic AE continuing beyond the 7-day period after vaccination.
- Solicited local or systemic AE that meets the definition of a SAE (as per Section 11.1.2).

11.1.5.1 Solicited local AEs

The following local AEs at the injection/electroporation site will be solicited:

- Injection site pain (including injection site muscle pain)
- Injection site tenderness
- Redness/Erythema
- Induration/Swelling
- Bruising
- Pruritus

11.1.5.2 Solicited systemic AEs

The following systemic AEs will be solicited:

- Fever
- Malaise/Fatigue
- Myalgia (excluding injection site muscle pain)
- Arthralgia

- Headache
- Nausea

11.2 Collection, assessment and documentation of AE and SAE data

The Investigator or appropriately delegated Staff will be responsible for detecting, documenting and reporting adverse events according to the reporting requirements and within the time periods herein specified. Subjects should be instructed by the Investigator or Staff to report any AE that occurs during participation in the trial. At each visit, subjects will be questioned in an appropriate manner (open-ended and non-leading questions) as to elicit unbiased responses about the occurrence of unsolicited AEs.

11.2.1 *AEs recording period*

AEs will be collected throughout the entire study period. Any AE occurring from the time of informed consent obtainment to the time of the first vaccination will be classified as a "pre-dose event" and must be recorded in the CRFs. Any AE occurring after the first vaccination and until the study completion, either observed by the Investigator or delegated Staff or reported by the subject, must be recorded in the AE section of the CRF, whether or not the event is considered to be causally related to treatment (solicited AE should not be entered in the CRF unless meeting one of the criteria listed in Section 11.1.5). These events will be accurately described (symptoms, objective data, onset date and time, stop date and time, severity, causality, action taken, follow-up, and outcome) and evaluated in terms of seriousness and causality.

Additional reports are required for all SAEs and for pregnancy cases (see Sections 11.3.1 and 11.5.3 for reporting requirements).

At any time after subject completion of the study, in case the Investigators became aware of any SAE suspected by the Investigator to be related to the vaccination, she/he will notify Rottapharm Biotech following the procedure described in Section 11.3.1.

11.2.2 *Description of signs or symptoms*

Whenever possible, the Investigator will record a specific diagnosis for the event. If a diagnosis cannot be made or sign/symptoms add significant information on the occurred AE, the Investigator will record each sign or symptom separately. In case of SAEs involving different diagnoses or signs and symptoms, they will be recorded within the same SAE Report Form if the onset of the symptoms occurred within a reasonable time interval and/or they are considered by the Investigator as medically related.

If multiple episodes of an event occur, separated by an appropriate time interval to justify considering the subsequent episodes as a repeated occurrence, the Investigator will record each episode separately and will fill in one SAE Report Form for each event.

The Investigator will define whether the reported AE/SAE is an exacerbation/worsening or increased frequency/severity of a pre-existing condition, providing relevant details and referencing the medical history as documented at screening.

11.2.3 *Severity*

The severity of solicited local and systemic AEs will be assessed based on scales derived from the FDA guidance on toxicity grading scales for healthy adult volunteers enrolled in preventive vaccine clinical trial.[38]

Table 6. Local AEs Grading Scale

Solicited local AEs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Injection site pain	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room or hospitalization
Injection site tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room or hospitalization
Redness/Erythema**	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling***	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Pruritus	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room or hospitalization

* Bruising will be measured (cm) and recorded by the participant in the e-diary as such.
** Redness/Erythema will be measured (cm) and recorded by the participant in the e-diary, then categorized during analysis as absent, mild, moderate or severe according to the grading shown in the table. Redness/Erythema ≤2.5cm is an expected consequence of skin puncture and will not be considered an AE.
*** Induration/Swelling will be graded using both the functional scale and the actual measurement.

Table 7. Systemic AEs Grading Scale

Solicited systemic AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Fever (°C)*	37.5 – 38	38.1 – 39	39.1 – 40	> 40
Malaise/Fatigue	No interference with activity	Some interference with activity	Prevents daily activity	Emergency room or hospitalization
Myalgia	No interference with activity	Some interference with activity	Prevents daily activity	Emergency room or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Prevents daily activity	Emergency room or hospitalization
Headache	No interference with activity	Some interference with activity	Prevents daily activity	Emergency room or hospitalization
Nausea	No interference with activity	Some interference with activity	Prevents daily activity	Emergency room or hospitalization

*During the 7-day period after each vaccination, body temperature will be collected daily (in the evening), by the participant using a thermometer given by the study staff. Body temperature will be measured and recorded to 1 decimal place in the e-diary (the highest temperature per day), and then categorized during analysis according to the grading shown in the table.

Participants experiencing severe (Grade 3) solicited events are asked to contact the Investigator who should ascertain further details and determine whether an unscheduled visit is required (see Section 11.4.1). Only an Investigator or medically qualified person is allowed to classify a participant's local reaction or systemic event as potentially life-threatening (Grade 4). If a participant experiences a confirmed Grade 4 event, the Investigator must immediately notify the Sponsor.

Clinically significant abnormalities in vital signs will be graded for AE using the FDA toxicity grading scales for healthy volunteers enrolled in vaccine clinical trials[38] (see Appendix I).

The severity of the AEs not listed in the tables above will be graded according to the following definitions:

Mild (Grade 1): A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate (Grade 2): A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe (Grade 3): A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Potentially Life Threatening (Grade 4)

AE will be captured in the CRF at the maximum severity reported.

The term "severe" is here used here to describe the severity (intensity) of the specific event; it is not the same as "serious", which is based on the occurrence of one or more of the seriousness criteria reported in Section 11.1.2.

11.2.4 *Causality*

An Investigator's causality assessment is the determination of whether there exists a reasonable possibility that the vaccination caused or contributed to an AE. The Investigator will evaluate the causal relationship between the study drug administration (injection and/or electroporation) and each occurred AE, and will record the results of this evaluation in the AE Report Form of the CRF, and in the SAE Report Form if applicable.

The Investigator will use his/her clinical judgement to determine the relationship and will make every effort to determine the cause of each AE. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, and other risk factors will be considered. The Investigator will also consult the Investigator's Brochure or other product information.

The relationship of an AE/SAE to the vaccination will be classified as follows, according to the binary decision approach recommended by the CIOMS VI Working Group:

Reasonable causal relationship
The expression "reasonable causal relationship" means that there are facts (evidence) or arguments to suggest a causal relationship.

No reasonable causal relationship

The Investigator will record the causality assessment and his/her "reason for judgment" in the AE Report Form of the CRF, and in the SAE Report Form if applicable.

Causality will be determined both by the Investigator and by Rottapharm Biotech. For regulatory purposes, the AE is classified as "related" if either the Investigator or Rottapharm Biotech determines that there is a reasonable suspected causal relationship to the study drug. However, the causality assessment given by the Investigator should not be downgraded by Rottapharm Biotech. If Rottapharm Biotech disagrees with the Investigator's causality assessment, both opinions will be provided with the report.
Causality could be provisionally classified as conditional/unclassified in case further data, essential for a proper assessment of the event, are under evaluation, but the final relationship should be provided as soon as possible. Unexpected SAEs, including laboratory test abnormalities, for which further data essential for a proper assessment are under evaluation, will be considered as a SUSAR until the assessment is completed.

11.2.5 *Outcome and subject follow-up*

The outcome of the AE will be classified as follows:

- recovered/resolved
- recovering/resolving
- recovered/resolved with sequelae
- not recovered/not resolved
- fatal
- unknown.

The Investigator will provide details for the latter 4 categories.

The Investigator will make every attempt to follow the subject until the AE/SAE is resolved or until acceptable stabilization in the event of chronicity.

11.3 Reporting SAE to Rottapharm Biotech

11.3.1 *SAE Reporting*

As soon as the Investigator becomes aware of an **SAE**, she/he will report **immediately—in any case no later than 24 hours after she/he becomes aware of it—**initial information by filling in, in capital letters and in English language, the SAE Report Form and sending a scanned copy to the dedicated e-mail address:

COV-1-2-01-SAE@rottapharmbiotech.com

In case of any need with respect to such reporting and/or to further information/instructions required, the Investigator may contact any of the following individuals with 24/7 availability, as provided in Table 8.

Table 8. Serious Adverse Event: contact personnel at Rottapharm Biotech

Name/Title	Office Telephone Number	Mobile Telephone Number
Federica Girolami, PharmD, MSc Director, Drug Safety e-mail: federica.girolami@rottapharmbiotech.com	+39-039-9066089	+39-346-4131428
Lucio Rovati, M.D. Chief Executive Officer/Chief Scientific Officer e-mail: lucio.rovati@rottapharmbiotech.com	+39-039-9066104	+39-335-202307

The SAE Report Form will be filled in according to the guidance for completion provided to the Investigator together with the SAE Report Form.

The Investigator will follow up the SAE by reporting relevant missing information (e.g. diagnosis, outcome, results of specific investigations). As soon as new or follow-up information are available, a new SAE Report Form with updated information will be filled in as per the same timeline described above (i.e. **no later than 24 hours after becoming aware of the new/follow-up information)**.

Additional documentation, if required, should be attached in a pseudonymised form to the SAE Report Form. In case of fatal outcome, the Investigator shall actively seek any additional requested information (e.g. autopsy reports and terminal medical reports). Pseudonymised and certified copy of the autopsy report should be sent to Rottapharm Biotech as soon as available and the Investigator should always provide a reason if the autopsy report cannot be provided (e.g. the family denied the consent). The Investigator will retain a certified copy of the autopsy report on-site with the study documentation.

For any SAEs, especially those that are fatal or life-threatening, lacking significant information or ongoing at the time of initial reporting, the Investigator should proactively seek follow-up information and send as follow-up SAE Report Form at her/his earliest convenience (in any case no later than 24 hours from awareness).

The Investigator must also report all SAEs in the CRF by filling in the AE Report Form.

A comprehensive narrative report of the case should be prepared by the Investigator, using the appropriate section ("Comments") of the SAE Report Form. The initial notification should include, at a minimum, the following information:

- an identifiable reporting source;
- a unique clinical trial identification;
- an identifiable subject (study subject identification code/number);
- a suspect investigational medicinal product;
- an adverse event assessed as serious.

The original SAE Report Form will be collected by the Clinical Research Associate at the monitoring visit after complete review of the case, and a certified copy is to be kept in the Investigator's Study File.

11.4 Measures to monitor participants safety

11.4.1 *Local and systemic Reactogenicity*

Participants experiencing any of the following from Day 1 (day of vaccination) to Day 7 are asked to contact the site staff or Investigator *immediately*, to determine if an unscheduled visit is required:

- Fever ≥ 39.0°C
- Redness/erythema at the injection site measuring greater than 10 cm
- Induration/swelling at the injection site measuring greater than 10 cm
- Severe pain at the injection site
- Severe tenderness at the injection site
- Any severe systemic event (including generalized pruritus).

Participants experiencing a medically attended event (e.g. doctor's visit, emergency room visit) or hospitalization are asked to contact the site staff or Investigator.

11.4.2 *Symptoms associated with COVID-19 and ADE monitoring*

Participants are asked to contact the site staff or Investigator *immediately* if at any time they experience:

- Respiratory symptoms, such as new or increased cough, shortness of breath, sore throat, wheezing, sputum production, nasal congestion, nasal discharge
- Symptoms that are associated with COVID-19 (e.g. body temperature ≥ 37.5 °C, loss of taste/smell).

If the Investigator will consider these symptoms compatible with COVID-19, the participant will be assessed for the presence of SARS-CoV-2 infection in respiratory tract samples. Suspect cases of COVID-19 will be treated according to local guideline until an infection with SARS-CoV-2 is not excluded by a molecular test.

The occurrence of disease enhancement following vaccination will be monitored. Participants with confirmed COVID-19 will be followed until clearance of the infection. Severe COVID-19 disease will be defined using clinical criteria. Detailed clinical parameters will be collected from medical records (including but not limited to: oxygen saturation, need for oxygen therapy, need for ventilatory support, imaging and blood test results).

11.5 Contraceptive measures and pregnancy

Women of childbearing potential should not be administered the vaccine used in this study until pregnancy is excluded.

In the present study, Investigators and subjects must adhere to the rules described in the following sections which have been written in accordance with the current "*Recommendations related to contraception and pregnancy testing in clinical trials*" of the Clinical Trial Facilitation Group (CTFG) established by the Heads of Medicines Agencies.[44]

11.5.1 *Definitions*

A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal, unless permanently sterile.
Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.
A postmenopausal state is defined as no menses for 12 months, without an alternative medical cause, in women who are at least 45 years of age.

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

11.5.2 *Contraceptive measures*

Prior to trial enrolment, all participants must be advised of the importance of avoiding pregnancy during trial participation and of the potential risk factors for an unintentional pregnancy. The subject must sign an Informed Consent Form documenting this discussion.

All subjects of childbearing potential and fertile men (with partner of childbearing potential) must adopt contraceptive measures from enrolment (i.e. after Informed Consent signature) up to study completion or at least 90 days after the last vaccination in case of withdrawal. Contraceptive measures allowed in this study are detailed below.

11.5.2.1 Women of childbearing potential

Women of childbearing potential will be included in the present study only after a confirmed menstrual period and a negative highly sensitive urine pregnancy test.

After Informed Consent signature, women of childbearing potential must use a highly effective method of contraception, i.e. one method out of the 7 listed below and acknowledged as such by the CTFG established by the Heads of Medicines Agencies:

1. Combined (oestrogen and progestogen containing) hormonal contraception with inhibition of ovulation as primary indication:
   - oral
   - intravaginal
   - transdermal.

   Subjects choosing this method should also use a supplementary male barrier contraceptive, i.e. *male condom*, because no interaction studies between the vaccine used in this study and contraceptive steroids are available to evaluate whether the efficacy of hormonal contraception is reduced.
2. Progestogen-only hormonal contraception with inhibition of ovulation as primary indication:
   - oral
   - injectable
   - implantable.

   Subjects choosing this method should also use a supplementary male barrier contraceptive, i.e. *male condom*, because no interaction studies between the vaccine used in this study and contraceptive steroids are available to evaluate whether the efficacy of hormonal contraception is reduced.

3. Intrauterine device (IUD).
4. Intrauterine hormone-releasing system (IUS).
5. Bilateral tubal occlusion.
6. Vasectomised partner (provided that partner is the sole sexual partner of the women participating the trial). If vasectomy has been performed (as documented) less than 3 months prior to study start, a supplementary method should be added e.g. male condom.
7. Sexual abstinence: sexual abstinence is acceptable only if this is the subject usual lifestyle and he/she is not likely to become sexually active during the entire period of risk, and it is thus further defined as refraining from heterosexual intercourse during the entire period of risk, i.e. from enrolment up to study completion or at least 90 days after the last vaccination in case of withdrawal.

The highly effective method of contraception should be documented in source documents.

11.5.2.2 Fertile men with women of childbearing potential

In the present study, fertile male subjects who are sexually active with women of childbearing potential must use *condom* and *refrain from donating sperm* from enrolment up to study completion or at least 90 days after the last vaccination in case of withdrawal.

11.5.2.3 Subjects exempted from using contraceptive measures

Female subjects of non-childbearing potential according to the definitions above do not need to use contraceptive measures.

Fertile male subjects who are sexually active with women of non-childbearing potential do not need to use contraceptive measures, provided that partner is the sole sexual partner of the man participating in the trial.

11.5.3 *Unintentional pregnancy*

Pregnancy testing will be performed at the study visits indicated in the flowchart and subjects will be advised that whenever one menstrual cycle is missed during the period of risk, i.e. after Informed Consent signature and up to study completion or 90 days after the last vaccination in case of withdrawal (or when potential pregnancy is otherwise suspected), they should perform a pregnancy test and contact the Investigator.

In the event of a pregnancy:

- If the pregnancy is diagnosed before the vaccine administration, the subject must not be vaccinated and must be withdrawn from the trial.
- If the pregnancy is diagnosed after the vaccine administration, the additional (boost) dose of vaccine (if any) must not be given and the subject must be withdrawn from the trial.

Subject must be instructed to report pregnancies to the Investigator, including pregnancies of a partner of a male trial participant, from the time of Informed Consent signature up to study completion or 90 days after the last vaccination in case of withdrawal. The Investigator is responsible for reporting the case by filling in both the SAE Report Form within 24 hours of first knowledge (see Section 11.3.1) and a specific Pregnancy Form (this

latter within 48 hours of first awareness date at the latest) regardless of whether an AE occurred or not.

All pregnancies will be followed until the final outcome is known. Pregnancy resulting in an abnormal outcome (e.g. congenital anomaly) should be monitored to follow the newborn development for an appropriate post-delivery period. If a pregnancy results in an abnormal outcome which the Investigator considers might be due to the study drug, this will be treated as an expedited report.

11.6 Reference document for expectedness assessment

The expectedness assessment is a Rottapharm Biotech's responsibility.

The reference document for expectedness assessment of SAEs related to COVID-*e*Vax for the present study is Section 6.4.8 of the COVID-*e*Vax IB in force at the time of SUSAR occurrence.

11.7 Safety issues and urgent safety measures

Events may occur during a clinical trial which do not fall within the definition of SUSAR and thus are not subject to the reporting requirements for SUSARs, even though they may be relevant in terms of subject safety. These events/observations are not to be reported as SUSARs, but they might require other actions, such as urgent safety measures, substantial amendments, or early termination of the trial. As reported in the "Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials of medicinal products for human use", herein referred to as CT-3 guidance, examples of safety issues other than SUSAR are:

- new events related to the conduct of a trial or the development of an IMP likely to affect the safety of subjects, such as:
  - ✓ a serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial,
  - ✓ a significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease,
  - ✓ a major safety finding from a newly completed animal study (such as carcinogenicity),
  - ✓ a temporary halt of a trial for safety reasons if the trial is conducted with the same investigational medicinal products in another country by the same sponsor,
- recommendations of the Independent Data Safety Monitoring Committee (IDSMC), if any, where relevant for the safety of subjects,
- in the case of advanced therapy investigational medicinal products, relevant safety information regarding the procurement or the donor.

Rottapharm Biotech will inform the concerned Competent Authority and the Ethics Committee of safety issues, which might materially alter the current benefit-risk assessment of the IMP while not falling within the actions listed above.

In case of occurrence of any new events relating to the conduct of the trial or the development of the IMP where the new event is likely to affect the safety of the subjects, Rottapharm Biotech and the Investigator shall take appropriate urgent safety measures to protect the subjects against any immediate hazard. Rottapharm Biotech shall forthwith inform the Competent Authorities of those new events and the measures taken and shall ensure that the Ethics Committee is notified at the same time.

Urgent safety measures may be taken without prior notification to the national Competent Authority. However, Rottapharm Biotech must inform ex post the national Competent Authority and the Ethics Committee of the Member State concerned of the new events, the measures taken and the plan for further action as soon as possible. The ex post notification of urgent safety measures is independent of the obligation to: notify substantial amendments, notify early termination of the trial, and notify adverse events and serious adverse reactions, as per current regulations.

11.8 Regulatory requirements for SUSARs and other safety issues

Rottapharm Biotech has the legal responsibility to notify the concerned Competent Authorities, the Ethics Committees and the Investigators concerned about SUSARs and other safety issues (e.g. whenever they may change the benefit-risk profile of the IMP or might require changes in the IMP development or in the overall conduct of the trial). For this study, reporting activities will be ensured by Rottapharm Biotech or the delegated CRO, as per the specific Safety Manual. Rottapharm Biotech will comply with country specific regulatory requirements relating to safety reporting to Regulatory Authorities, Ethics Committees and Investigators.

The Investigator who receives a safety notification describing a SUSAR or other safety issues from Rottapharm Biotech will file it with the IB into the Investigator's File.

11.9 Incidents/User errors/Complaints to the EP medical device

In this study the pulse generator Cliniporator® and the electrodes EPSGun described in Section 7.5 will be used to perform the electroporation.

Adverse events related to the use of the medical device will be collected as described in Section 11.1, 11.2 and 11.3 above.

In addition:

- any malfunction or deterioration in the characteristics and/or performance of a device, as well as any inadequacy in the labeling or the instructions for use
- any use error (i.e. an act or omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user)
- any complaint to the use of the medical device

will be collected by the Investigators throughout the study and reported to the Sponsor using a dedicated Incident/User error/Complaint Report Form that will be filled in, scanned and sent to the same e-mail address indicated in Section 11.3.1.

If one of the above circumstances might lead, directly or indirectly, or might have led to death of a patient, user or of other persons or to a serious deterioration in their state of

health, the Investigator shall report to Rottapharm Biotech this occurrence **immediately—in any case no later than 24 h after awareness.** All the other occurrences shall be reported to Rottapharm Biotech within 3 days of awareness by the Investigator.

All incidents will be reported by the Sponsor to the manufacturer of the medical device used in this study (i.e. IGEA) which will inform the relevant Competent Authority as per current regulations on medical devices.

12. DATA ANALYSIS AND STATISTICAL METHODS

In agreement with the most recent EMA guideline on "Clinical evaluation of new vaccines"[36] and recently published early studies investigating the effects of candidate vaccines against SARS-CoV-2, this Phase I/II study is not designed to test a specific formal statistical hypothesis, but rather to obtain preliminary estimates on the safety, reactogenicity and immunogenicity of COVID-*e*Vax.

Following is a brief description of the general strategy that will be adopted for the statistical analysis. A fully detailed Statistical Analysis Plan will be developed and released prior to database lock.

All recorded and calculated data will be presented in tables and listings. Standard procedures depending on the underlying distribution will be used. Descriptive statistics for ordinal and categorical variables will be expressed as numbers and percentages whereas, for continuous variables, n (non-missing sample size), mean, standard deviation, median, maximum and minimum will be shown, unless otherwise noted in the SAP. Any missing data will not be imputed.

For all parameters the last value measured before the first COVID-*e*Vax dose administration is defined as Baseline.

12.1 List of statistical objectives and endpoints

12.1.1 *Statistical objectives*

12.1.1.1 Phase I (Dose Escalation)

Primary objective

- To assess the safety and reactogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To identify the dose(s)/schedule(s) to be used in the Phase II (Dose Expansion)

Secondary objectives

- To preliminarily assess the immunogenicity of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

12.1.1.2 Phase II (Dose Expansion)

Primary objective

- To assess the immunogenicity of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

Secondary objectives

- To assess the duration of the immune response of the selected dose(s)/schedule(s) of the candidate vaccine COVID-*e*Vax in healthy adult volunteers
- To assess the (long-term post-administration) safety of the candidate vaccine COVID-*e*Vax in healthy adult volunteers

12.1.2 *Endpoints*

12.1.2.1 Phase I - Primary endpoint

Safety:

- Reactogenicity:
  - Incidence of solicited local AEs at the injection site
    (time frame: through 7 days post-each vaccination)
  - Incidence of solicited systemic AEs
    (time frame: through 7 days post-each vaccination)

- Incidence of unsolicited AEs
  (time frame: through 4 weeks post-each vaccination)

- Changes in safety laboratory parameters
  (time frame: through 4 weeks post-each vaccination)

12.1.2.2 Phase I - Secondary Endpoints

Immunogenicity
(time frame: through 4 weeks post-last vaccination)

- Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen, analysed as GMT and GMFR from baseline
- SARS-CoV-2 neutralizing antibody titer, analysed GMT and GMFR from baseline
- Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2 as determined by IFN-γ ELISpot
- Percentage of participants who seroconverted
- Duration of the immune response on all criteria and parameters used as secondary endpoint (time frame: through study completion)

- Incidence of unsolicited AEs through study completion (6 months)

All primary and secondary endpoints will also be assessed in the time frame through study completion (6 months).

12.1.2.3 Phase II - Primary endpoints

Immunogenicity
(time frame: through 4 weeks post-last vaccination):

- Quantitative antibody titers, binding to the specific SARS-CoV-2 antigen, analysed as GMT and GMFR from baseline
- SARS-CoV-2 neutralizing antibody titer, analysed as GMT and GMFR from baseline

- Change from baseline in antigen-specific cellular immune responses to SARS-CoV-2 as determined by IFN-γ ELISpot
- Percentage of participants who seroconverted

12.1.2.4 Phase II - Secondary endpoints

Immunogenicity
(time frame: through study completion at 6 months post-first vaccination):

- Duration of the immune response on all criteria and parameters used as primary endpoint

Safety

Reactogenicity:
- Incidence of solicited local AEs at the injection site(s)
  (time frame: through 7 days post-each vaccination)
- Incidence of solicited systemic AEs
  (time frame: through 7 days post-each vaccination)

- Incidence of unsolicited AEs
  (time frame: through 4 weeks post-each vaccination)

- Changes in safety laboratory parameters
  (time frame: through 4 weeks post-each vaccination)

- Incidence of unsolicited AEs through study completion (6 months)

All primary and secondary endpoints will also be assessed in the time frame through study completion (6 months).

12.2 Rationale for sample size

No formal sample size calculation has been performed. The present open label Phase I/II study is designed to assess the safety, reactogenicity, and preliminary immunogenicity of COVID-*e*Vax, balancing the safety of volunteers with the aims of assessing the vaccine safety profile and immunogenicity after selected dosing schedules of the candidate vaccine.

In Phase I, 20 subjects for each dose schedule group will be enrolled. Twenty subjects per group are considered sufficient to assess safety and reactogenicity, while preliminarily assessing immunogenicity. This sample size will give a 88% chance of observing at least 1 AE, in case of AEs with 10% underlying incidence, and a 90% chance in case the underlying incidence is 20%. If no AE is observed among 20 subjects, this will provide an 80% confidence that the underlying incidence rate is < 7.7% or a 90% confidence that the underlying rate is < 10.8%.

In Phase II, 80 subjects will be included in each expanded dose schedule cohort. This number is considered sufficient to characterize the safety and immunogenicity of each expanded dose schedule cohort, that would be evaluated in a total of 100 subjects each (considering both study phases). Under seroconversion rates of 70% and 90%, the width of the estimated 95% Confidence Interval (based on a Binomial "exact" calculation) will be 19% and 13%, respectively.

12.3 Data management

The Investigator will enter data for each participant involved in the study into an electronic CRF (eCRF) designed to record all the protocol-required information; the Investigator should ensure the accuracy, completeness, and timeliness of the data reported in the eCRF as well as the consistency with Source Documents.

Data collected in the eCRF will be entered into a validated database system. A combination of manual and programmatic edit checks will be used to review the data for completeness, logic and adherence to study protocol. As a result of these checks, queries may be issued to the investigational sites.

Data entered into the e-diary by the participants will be transferred electronically into the same validated database system, and will be available for review by the Investigators via the web-based portal of the eCRF. Diary entries are not source data and will not be submitted to any source data verification procedures.

When all data have been reviewed, all specific requirements have been satisfied and the SAE reconciliation has been completed and approved, the database will be locked. Details on the Data Management process will be provided in the Data Management Plan.

12.4 Study populations

- Screened Population: all participants who sign their written informed consent.
- Intention-To-Treat (ITT) Population: all participants who received at least one dose of the vaccine and contributed both pre- and at least one post-vaccination venous blood samples for immunogenicity testing for which valid results are reported.
- Per Protocol (PP) Population: all participants included in the ITT population who completed the study with no major protocol deviations.
- Safety Population: all participants who received at least one dose of the vaccine.

12.5 Demographics and other baseline characteristics

Demographic (age, sex, race/ethinicity, BMI) and baseline characteristics will be reported in tables and listings by cohort.

The main analyses may be stratified by demographic or other baseline characteristics, if relevant.

12.5.1 *Medical history*

The Medical Dictionary for Regulatory Activities (MedDRA) will be used for coding the participant's medical history. For each SOC and PT, the number and percentage of participants reporting abnormalities in medical history will be summarized by cohort.

12.5.2 *Prior and concomitant medications*

The WHO Drug dictionary will be used to classify medications by therapeutic class, and the MedDRA dictionary will be used for coding the relevant reason for medication. Further coding will be performed using the Anatomical Therapeutic Chemical (ATC) classification system. Prior medication is defined as medication administered in the month preceding the screening. Concomitant medication is defined as medication taken between the day of the first vaccination and the day of last visit. Medications taken between the day of Informed Consent signature and the day of the first vaccination will be classified as Pre-Dose medications. Prior/concomitant medications will be summarized as the number and percentage of participants (by cohort) receiving each drug within each therapeutic class.

12.5.3 *Physical examination*

Findings observed at physical examination will be recorded in the medical history or as adverse events (see Section 9.2.3), and as such summarized and listed.

12.5.4 *12-lead ECG*

As for 12-lead ECG, results collected at baseline will be tabulated by cohort.

12.6 Primary and Secondary Analyses

Reactogenicity, safety and immunogenicity will be considered as primary or secondary endpoints, depending on the phase of the Study, being reactogenicity and safety the primary endpoints for Phase I and immunogenicity the primary endpoint for Phase II.

As the analyses will be conducted according to the same methodologies regardless whether or not constitute the primary or the secondary endpoint analyses, they will be described once, with the safety and reactogenicity analyses decribed in Section 12.6.1 and the immunogenicity analyses described in Section 12.6.2, respectively.

12.6.1 *Safety and Reactogenicity Analyses*

The analysis of safety and reactogenicity data will be carried out on the Safety Population. Reactogenicity and safety data analysed through week 4 post last vaccination will be the primary endpoint analyses for Phase I.

12.6.1.1 Adverse events

AEs will be coded using the MedDRA dictionary.

All Treatment Emergent Adverse Events (TEAEs) will be included in the analysis. A TEAE is an AE as defined in Section 11.1.1, occurring after the vaccine administration.

Analysis of Reactogenicity

It will be based on the incidence of solicited local AEs at the injection site and solicited systemic AEs, summarized by severity for each day post vaccination (Days 1-7) and as the maximum severity over all 7 days.

Analysis of Safety

Unsolicited AEs will be collected from the time of first vaccination through the end of study (Day 169) and will be summarized by cohort, for the first 28 days after vaccination and then for the overall study period.

Unsolicited AEs will be summarized as number and percentage of participants reporting at least one event in each MedDRA SOC, MedDRA PT, and cohort, cross tabulated by severity and relationship to study product. Additionally, the proportion of participants and exact 95% CIs of AEs in aggregate and by MedDRA categories will be computed. Within a specific category, when the participant has more than one event reported, she/he will be counted only once for each category.

SAEs and AEs leading to discontinuation will be reported in detailed listings showing the event description, MedDRA SOC, MedDRA PT, and cohort, relevant dates (vaccinations and AEs), severity, relationship to study product, and outcome for each event.

12.6.1.2 Laboratory data

Routine hematology and clinical chemistry and pregnancy test will be performed for safety reasons at screening, at selected time points and at the end of trial.

For laboratory data, descriptive statistics will be reported by cohort and study visits. Out-of-range data reported as CS by the Investigator will be summarized. Moreover, the number and percentage of participants with CS out-of-range values will be calculated for participants with baseline and a post-baseline value.

Graphical presentations may include box plots and shift plots.

12.6.1.3 Vital signs

As far as vital signs (i.e. blood pressure, heart rate, respiratory rate, body temperature) are concerned, descriptive statistics on raw data as well as on changes between data collected at each visit and baseline will be presented by cohort.

12.6.2 *Immunogenicity analysis*

The analysis of immunogenicity data will be carried out on the ITT population. If there are protocol deviations which may affect the results, the analysis may also be performed on the PP Population. The analysis of immunogenicity data will be the primary endpoint for Phase II.

Quantitative humoral responses will be expressed as antibody titers (binding and neutralizing), and analysed as GMT and GMFR from baseline.

Seroconversion is defined as having an antibody level above the lower limit of quantification (LLOQ) post vaccination if the baseline level was below the LLOQ, or a 4-fold increase over baseline post vaccination if the baseline level was above the LLOQ. Seroconversion rates of both binding and neutralizing antibodies, GMFR and GMT, will be calculated at Day 1 (GMT only) and at specific post-baseline timepoints by cohort and will be summarized graphically.

Seroconversion rates of both binding and neutralizing antibodies, GMFR and GMT will be presented with their corresponding 95% confidence interval (CI) estimates at each

timepoint and overall peak GMT, and the pair-wise differences between seroconversion rates by cohort will be summarized by study day along with 95% CIs.

The vaccine induced T-cell response will be summarized at each timepoint by cohort, and will be displayed graphically.

Regression models on immunogenicity data may also be conducted.

Comparison of any or all the above vaccine-specific humoral and cellular responses vs responses after natural infection (i.e. with responses from vaccine-naïve individuals who have recovered from natural SARS-CoV-2 infection) will be also carried out.

12.7 Handling of protocol deviations

In order to identify participants in the PP population, it will be necessary to define which variations from the protocol will be considered major and which minor protocol deviations according to a pre-defined list.

All protocol deviations occurring during the study period will be summarized by treatment group.

12.8 Timelines of the planned analyses

The final analyses will be performed and clinical study report (CSR) completed when all primary and secondary safety and immunogenicity endpoint data are available. The CSR will be completed after the final data lock (through Day 169).

Early interim analyses of safety, reactogenicity, and immunologic response data are planned once all participants (Cohorts 1-4) have reached the following predefined milestones and the relevant data are entered in the database and validated:

- **Upon availability of safety and immunogenicity data (4-week) for Phase I (key decision point to move to Phase II)**
- **Upon availability of safety and immunogenicity data (4-week) for Phase II (key decision point to move to Phase III)**
- **Upon availability of 12-week data for Phase II**

Cumulative safety information, study status, and primary endpoint results may be presented at a public forum as summaries aggregated by study arm or as individual data, at the discretion of the Sponsor or the Partner while the study is ongoing. None of the interim analyses will include any formal statistical hypothesis testing, therefore, p value adjustment will not be made to any analyses.

13. ETHICS

This study will be conducted in accordance with the ethical principles outlined in the current version of the "Declaration of Helsinki", with ICH-GCP guideline, and with all applicable regulatory requirements. Prior to initiation of the study in each study site, Rottapharm Biotech will obtain approval from the appropriate Regulatory Authority(ies) to conduct the study in accordance with ICH-GCP and all the applicable country-specific regulatory requirements.

13.1 Independent Ethics Committee (IEC)

This protocol and the informed consent document will be submitted to the appropriate IEC(s) for written approval.

Rottapharm Biotech may not start the study until the IEC has issued a favourable opinion. A listing of the members of the Committee is requested; the Investigator is not allowed to participate in the Committee's decision.

In case of study amendments, the IEC shall be notified according to the procedure described in Section 14.8 and, if the case (i.e. substantial amendments), the IEC shall give an opinion in due time before the amendment may be implemented.

The Investigator will agree to submit any required progress reports to the IEC and any additional requested information (e.g. for reported deaths). Rottapharm Biotech shall ensure that relevant information about SUSARs, as well as any other safety issue, is reported to the IEC in due time according to regulatory requirements. In addition, once a year throughout the study, Rottapharm Biotech shall provide the IEC with a report of all suspected serious adverse reactions which have occurred over that period and a report of the participants' safety. The IEC will be notified by the Investigator about the study termination.

13.2 Subject information and consent

Prior to study participation, the participant will be informed of the nature of the vaccine and will be given pertinent information as to the intended purpose, procedures and possible benefits and risks of participation in the study. A Participant Information Sheet, including information about the study and a written Informed Consent Form, will be provided by Rottapharm Biotech and approved by the relevant IEC. The document must be in compliance with ICH-GCP and in accordance with local regulatory and legal requirements, and should be written in language readily understood by the participant. After reading the informed consent document, participants who are willing to participate in the study must give consent in writing. The Informed Consent Form must be signed and **personally dated** by the participant and by the person who conducted the informed consent discussion. The participant will be provided with a certified copy of both the information sheet and the signed consent form.

The Investigator will not undertake any study specific procedure until a valid consent has been obtained.

The original signed consent document will be retained by the Investigator. A statement that signed informed consent has been obtained will be noted on the study CRF.

13.3 Subject confidentiality

The Investigator must ensure that participant confidentiality is maintained. On the CRFs and other study documents provided to Rottapharm Biotech, or anyhow outside the investigational site, the participants should be identified only by their identification code. Source Documents should be kept in strict confidence by the Investigator.

In compliance with ICH-GCP guideline, it is required that the Investigators/Institution ensure direct access to original source documentation to authorized representatives of Rottapharm Biotech (CRAs and auditors), of the Regulatory Authorities, and of the EC for verification of study-related procedures and data. Participants will be informed that the above-named representatives should review their study-related records without violating the confidentiality.

14. STUDY PROCEDURES

14.1 Independent Data Safety Monitoring Committee

An Independent Data Safety Monitoring Committee (IDSMC), composed of three members (inclusive of the IDSMC Chair), experts in or representatives of the fields of immunology, infectious diseases, clinical trials and biostatistics, will be instituted.

The IDSMC is responsible for safeguarding the interest of trial participants, for assessing the safety of the interventions during the trial, and for protecting the scientific validity of the study.
The IDSMC is responsible for providing the Sponsor and the Partner, who are in charge of the final decision, with recommendations related to the dose-escalation and the dose-expansion. In addition, in order to protect subject's safety, the IDSMC is responsible for providing recommendations relevant to trial interruption or study protocol amendments.

14.2 Procedures for Safety Review and Dose Escalation

Dose-escalation during Phase I and dose expansion in Phase II will be evaluated at the Safety Review/Dose Escalation meetings by the IDSMC.

Telephone conferences/meeting will be scheduled weekly or more frequently if needed in the dose escalation phase in order to review overall safety data from the specific cohort and from prior cohorts (if applicable), and to agree on recommendations on dose escalation/dose expansion.

In the dose-expansion phase the safety review meeting will be held monthly.

Ad hoc meetings can also be scheduled at any time, if deemed necessary by the IDSMC.

14.3 Quality Control and Quality Assurance

14.3.1 *Monitoring*

The study will be monitored by Rottapharm Biotech's CRA designee, in compliance with relevant SOPs, ICH-GCP guideline and all applicable regulatory requirements. The CRA will act as the main line of communication between Rottapharm Biotech and the Investigational Site. The main CRA responsibility is to ensure that the trial is properly conducted and documented.

To achieve this goal, the CRA will carry out the following visits:

- Trial Initiation Monitoring Visit aimed at ensuring the Principal Investigator and the Staff are properly instructed on how to follow the study protocol and to conduct the trial; this assumes all required essential documents for the trial have been obtained and the IMPs are available at Site.
- Monitoring Visits aimed at verifying that the rights and well-being of human participants are protected; the reported trial data are accurate, complete, and verifiable from source documents; the conduct of the trial is in compliance with

the currently approved protocol/amendments, with ICH-GCP, and with all the applicable regulatory requirements.

- Close-out Monitoring Visit aimed at ensuring essential documents are available; arrangements for retention of study and source data have been made; all remaining unused supplies are returned and/or destroyed.

The study monitoring will be conducted according to a risk-based approach, as described in the Clinical Monitoring Plan.

14.3.2 *Auditing*

The study may be subject to audits by the Sponsor or the Partner; audits are aimed at verifying whether the study is conducted and data are obtained according to the study protocol, SOPs, ICH-GCP guideline and all the applicable regulatory requirements.

In addition, representatives from Regulatory Authorities, (including foreign authorities) and IEC(s) may conduct regulatory inspections at any time during or after completion of the study; the Investigator has to immediately notify Rottapharm Biotech of any such inspection.

In the event of an audit or inspection, the Investigator/Institution will grant the auditors and the inspectors the direct access to source document and all trial-related files and correspondence, as well as access to site facilities and time to discuss any findings/relevant issues.

14.4 Early termination of the study

Rottapharm Biotech reserves the right to temporarily halt or terminate the study, as a whole or at a specific site, at any time for reasons including (but not limited to) urgent safety measures, ethical issues, substantial amendment or severe non-compliance. Rottapharm Biotech will discuss reasons for halt/premature termination with Investigators/Institutions (as applicable), providing advance notice of the termination when feasible, and will promptly inform the relevant Regulatory Authorities as required by the applicable regulations.

The clinical trial may also be suspended by Regulatory Authorities where they have objective grounds for considering that the conditions in the request for authorization are no longer met or in case of new information raising doubts about trial safety or scientific validity.

14.5 Clinical Study Report

At completion of each planned early interim analysis as detailed in Section 12.8 a report will be provided.

At the end of the study, an integrated (clinical and statistical) CSR will be prepared and provided according to the standards of the ICH Guideline for Structure and Content of CSRs.

Signatures of Coordinating Investigator(s) and/or Principal Investigator(s), depending on the Regulatory Authority's requirement, will be obtained. The Investigators will be provided reasonable access to statistical tables, figures, and a full summary of the study results. Rottapharm Biotech will submit, according to the applicable regulatory requirements, a clinical trial summary report, no later than 12 months after the last participant last visit.

14.6 Record retention

The Investigator/Institution should ensure proper maintenance of study essential documents, as defined in Section 8 of the ICH-GCP Guideline (i.e. filed at the Investigator/Institution sites), in a safe and secure location. Essential documents must be easily accessible in case of audit and regulatory inspections.

Rottapharm Biotech should obtain the Investigators/Institutions' agreement to retain the trial related essential documents until Rottapharm Biotech informs, in writing, the Investigators/Institutions that these documents are no longer to be retained. By signing this protocol, Rottapharm Biotech and the Investigators/Institutions provide confirmation of this agreement.

The Investigators/Institutions must notify Rottapharm Biotech of any changes in archival arrangements, including, but not limited to, archival of records at an off-site facility or transfer of ownership.

14.7 Insurance coverage and compensation to subjects and Investigators

Rottapharm Biotech will provide liability insurance which covers health impairments resulting from drugs administered in the course of the study for which the participant has given his/her written informed consent to participate. This liability insurance also covers health impairments resulting from measures carried out on the body of the person, on the condition that such measures are in connection with this study; that the Principal Investigators and assistants have followed the instructions of Rottapharm Biotech; and that the Principal Investigators and assistants have, in general, performed the trial correctly and in accordance with accepted scientific procedures and with the protocol.

14.8 Amendments to this Protocol

Any changes to the approved version of this study protocol will require a formal amendment to the protocol.

Substantial amendments (i.e. those that are likely to have an impact on the safety of trial participants or to change the interpretation of the scientific documents in support of the conduct of the trial, or if they are otherwise significant) will be agreed upon by Takis, Rottapharm Biotech and the Investigators. Rottapharm Biotech or delegated responsible shall notify the Competent Authority of the Member State(s) and the EC(s) concerned. Substantial amendments may be implemented only when the favourable opinion of the

EC(s) concerned has been obtained and the Competent Authority of the Member State(s) has/have raised no grounds for non-acceptance.

Non-substantial amendments, although formally documented and agreed, do not have to be notified to IEC(s).

15. REFERENCES

[1] Chan JF-W, Yuan S, Kok K-H, et al.
A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster.
Lancet 2020; 395: 514–23.

[2] Wu F, Zhao S, Yu B, et al.
A new coronavirus associated with human respiratory disease in China [published correction appears in Nature. 2020 Apr;580(7803):E7].
Nature. 2020;579(7798):265-269. doi:10.1038/s41586-020-2008-3.

[3] World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. Published: 11 Mar 2020. Accessed: 01 Apr 2020.

[4] World Health Organization. WHO Coronavirus disease (COVID-19) pandemic update. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019?gclid=Cj0KCQjw3ZX4BRDmARIsAFYh7ZLM3CocY3sphcfUPoaGN1gFh90mStBu6hCZrwTX5PKix0z6shnj6qEaAvB6EALw_wcB Accessed: 01 September 2020

[5] Lurie N, Saville M, Hatchett R, Halton J.
Developing Covid-19 Vaccines at Pandemic Speed.
N Engl J Med [Internet]. 30 marzo 2020;382(21):1969–73.
Available at: https://doi.org/10.1056/NEJMp2005630.

[6] World Health Organization. Draft landscape of COVID-19 candidate vaccines. Available from: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines. Accessed: 27 October 2020

[7] Wrapp D., Wang N., Corbett KS et al.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science (80). 367, 1260–1263 (2020).

[8] Yuan, M. *et al.*
A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV.
Science 368, 630–633 (2020).

[9] Iwasaki, A., Yang, Y. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 20, 339–341 (2020). doi:10.1038/s41577-020-0321-6

[10] COVID-*e*Vax Investigator Brochure Edition 1.0, 2020.

[11] Gary EN and Weiner DB
DNA vaccines: prime time is now.
Current Opinion in Immunology 200, 66: 21-27.

[12] Martin, J. E., M. K. Louder, L. A. Holman, et al
A SARS DNAvaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial
Vaccine, 26: 6338-43, 2008

[13] Modjarrad, K., C. C. Roberts, K. T. Mills, A. R.
Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial
Lancet Infect Dis, 19: 1013-22. 2019

[14] S.H.T.JorritsmaE.J.GowansB.Grubor-BaukD.K.Wijesundara
Delivery methods to increase cellular uptake and immunogenicity of DNA vaccines
Vaccine Volume 34, Issue 46, 4 November 2016, Pages 5488-5494

[15] Khan A.S., Broderick K.E., Sardesai N.Y. (2014)
Clinical Development of Intramuscular Electroporation: Providing a "Boost" for DNA Vaccines.
In: Li S., Cutrera J., Heller R., Teissie J. (eds) Electroporation Protocols. Methods in Molecular Biology (Methods and Protocols), vol 1121. Humana Press, New York, NY – pages 279-289

[16] Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH (1982).
Gene transfer into mouse lyoma cells by electroporation in high electric fields
The EMBO Journal. 1 (7): 841–5. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC553119). PMID 6329708 (https://pubmed.ncbi.nlm.nih.gov/6329708).

[17] C Chang, Donald C. (2006-09-15), "Electroporation and Electrofusion", in Meyers, Robert A. (ed.), Encyclopedia of Molecular Cell Biology and Molecular Medicine, Wiley-VCH Verlag GmbH & Co. KGaA, doi:10.1002/3527600906.mcb.200300026 (https://doi.org/10.1002%2F3527600906.mcb.200300026), ISBN 9783527600908.

[18] Mir LM, Belehradek M, Domenge C, et al
L'électrochimiothérapie, un nouveau traitement antitumoral: premier essai clinique [Electrochemotherapy, a new antitumor treatment: first clinical trial].
C R Acad Sci III. 1991;313(13):613-618 (https://pubmed.ncbi.nlm.nih.gov/1723647).

[19] MacGregor RR, Boyer JD, Ugen KE, et al.
First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response.
J Infect Dis 1998; 178: 92–100.

[20] Flingai S, Plummer EM, Patel A, et al.
Protection against dengue disease by synthetic nucleic acid antibody prophylaxis/immunotherapy.
Sci Rep 2015; 5: 12616.

[21] Morrow MP, Tebas P, Yan J, et al.
Synthetic consensus HIV-1 DNA induces potent cellular immune responses and synthesis of granzyme B, perforin in HIV infected individuals.
Mol Ther 2015; 23: 591–601.

[22] Cashman KA, Wilkinson ER, Shaia CI, et al.
A DNA vaccine delivered by dermal electroporation fully protects cynomolgus macaques against Lassa fever.
Hum Vaccin Immunother 2017;13:2902–2911.

[23] Dowd KA, Ko SY, Morabito KM, et al.
Rapid development of a DNA vaccine for Zika virus.
Science 2016; 354: 237–240.

[24] Patel A, DiGiandomenico A, Keller AE, et al.
An engineered bispecific DNA-encoded IgG antibody protects against Pseudomonas aeruginosa in a pneumonia challenge model.
Nat Commun 2017; 8: 637.

[25] NIH U.S. National Library of Medicine ClinicalTrials.gov.
A Study to Test the Tolerability of an Investigational Electroporation Device in Healthy Adults (V930-001)
ClinicalTrials.gov Identifier: NCT00721461. https://clinicaltrials.gov/ct2/show/ NCT00721461

[26] Diaz CM, Chiappori A, Aurisicchio L, et al
Phase 1 studies of the safety and immunogenicity of electroporated HER2/CEA DNA vaccine followed by adenoviral boost immunization in patients with solid tumors
Journal of Translational Medicine 2013, 11:62 http://www.translational-medicine.com/content/11/1/62

[27] Aurisicchio L, Fridman A, Mauro D, et al
Safety, tolerability and immunogenicity of V934/V935 hTERT vaccination in cancer patients with selected solid tumors: a phase I study
J Transl Med 2020; 18:39 https://doi.org/10.1186/s12967-020-02228-9

[28] NIH U.S. National Library of Medicine ClinicalTrials.gov.
A First-In-Human Study to Evaluate Safety, Tolerability, Reactogenicity, and Immunogenicity of JNJ-64300535, a DNA Vaccine, Administered by Electroporation-Mediated Intramuscular Injection, in Participants With Chronic Hepatitis B Who Are on Stable Nucleos(t)Ide Therapy and Virologically Suppressed
ClinicalTrials.gov Identifier: NCT03463369. https://clinicaltrials.gov/ct2/show/ NCT03463369

[29] Diehl MC, Lee JC, Daniels SE, Tebas P, Khan AS, Giffear M, et al.
Tolerability of intramuscular and intradermal delivery by CELLECTRA® adaptive constant current electroporation device in healthy volunteers.
Hum Vaccines Immunother. 2013;9(10):2246–52.

[30] Supplement to: Modjarrad K, Roberts CC, Mills KT, et al.
Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial.
Lancet Infect Dis 2019; published online July 24.http://dx.doi.org/10.1016/S1473-3099(19)30266-X.

[31]FDA Guidance for Industry - Considerations for Plasmid DNA Vaccines for Infectious Disease Indications November 2007

[32] Liu L, Wei Q, Lin Q, et al.
Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection.
JCI insight 2019; 4(4).

[33] Tseng CT, Sbrana E, Iwata-Yoshikawa N, et al.
Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.
PLoS One 2012; 7(4): e35421.

[34] Weingartl H, Czub M, Czub S, et al.
Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets.
J Virol 2004; 78(22): 12672-6.

[35] Agrawal AS, Tao X, Algaissi A, et al. Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.
Hum Vaccin Immunother 2016; 12(9): 2351-6.

[36] Committee on Human Medicinal Products (CHMP).
Guideline on clinical evaluation of vaccines (draft).
EMEA/CHMP/VWP/164653/05, 26 April 2018

[37] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evalkuation and Research
Development and Licensure of Vaccines to prevent COVID-19, June 2020

[38] US Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research.
Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials, September 2007. http://www.fda.gov/cber/guidelines.htm (accessed May 30, 2020).

[39] Verschoor CP, Singh P, Russell ML, et al.
Microneutralization assay titres correlate with protection against seasonal influenza H1N1 and H3N2 in children.
PLoS One 2015; 10(6): e0131531.

[40] Kulkarni PS, Hurwitz JL, Simões EAF, Piedra PA.
Establishing correlates of protection for vaccine development: considerations for the respiratory syncytial virus vaccine field.
Viral Immunol 2018; 31: 195-203.

[41] Okba NMA, Müller MA, Li W, et al.
Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients.
Emerging Infect Dis 2020; 26: 1478-88.

[42] Yu J, Tostanoski LH, Peter L, et al.
DNA vaccine protection against SARSCoV-2 in rhesus macaques.
Science 2020 May 20 (Epub ahead of print) 10.1126/science.abc6284 (2020).

[43] Chandrashekar A, Liu J, Martinot AJ, et al.
SARS-CoV-2 infection protects against rechallenge in rhesus macaques.
Science 2020 May 20 (Epub ahead of print).

[44] Clinical Trial Facilitation Group (CTFG) Recommendations related to contraception and pregnancy testing in clinical trials, Final version 2014, pages 1-13.

LIST OF APPENDICES

APPENDIX I

Adapted from FDA severity grading criteria for physical observations

Vital Signs*	**Grade 1 (mild)**	**Grade 2 (moderate)**	**Grade 3 (severe)**	**Grade 4 Potentially Life threatening**
Tachycardia (bpm)	101 - 115	116 – 130	>130	Emergency room or hospitalisation for arrhythmia
Bradycardia (bpm)**	50 – 54	45 – 49	<45	Emergency room or hospitalisation for arrhythmia
Systolic hypertension (mmHg)	141 - 150	151 – 155	≥155	Emergency room or hospitalization for malignant hypertension
Diastolic hypertension (mmHg)	91 - 95	96 – 100	>100	Emergency room or hospitalization for malignant hypertension
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80	Emergency room or hospitalization for hypotensive shock
Respiratory Rate (breaths per minute)	17 - 20	21-25	>25	Intubation

**Taken after ≥10 minutes at rest*

***When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes.*

****Only if symptomatic (e.g. dizzy/ light-headed)*