Universitätsklinikum
Tübingen

P-pVAC-SARS-CoV-2: Phase I single-center safety and immunogenicity trial of multi-peptide vaccination to prevent COVID-19 infection in adults

Statistical Analysis Plan (SAP)

Version: Final

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Date: 28.06.2021 [redacted]

Review:

Date: 28.06.2021 [redacted]

Sponsor (represented by):

Date: 29.06.2021 [redacted]

Confidentiality statement

Note

This statistical analysis plan was established according to the SOP BI03 (17.05.2019) of the ZKS Tübingen. The analysis tables and listings will be independently validated by a second statistician according the SOP BI06 of the ZKS Tübingen.

Content

Abbreviations

ADR	Adverse Drug Reaction
ADE	Antibody-dependent Enhancement
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Transaminase (SGPT)
AP	Alkaline Phosphatase
aPTT	Partial Thromboplastin Time
AST	Aspartate Transaminase (SGOT)
BMI	Body Mass Index
CCR	Cellular Conversion Rate
CNS	Central Nervous System
COVID-19	Coronavirus Disease 2019
COV	Coronavirus
CMV	Cytomegalovirus
CNS	Central nervous system
CoVac-1	SARS-CoV-2-derived multi-peptide vaccine
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRP	C-reactive Protein
CTC(AE)	Common Toxicity Criteria (for Adverse Events)
DBL	Data Base Look
DSMB	Data and Safety Monitoring Board
EC	Ethic Committee
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form
EOS	End of Study
EOSf	End of Safety follow-up
FCBP	Female of Child Bearing Potential
γ-GT	Gamma Glutamyltransferase
GFR	Glomerular Filtration Rate
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen System
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LDH	Lactate Dehydrogenase
NYHA	New York Heart Association
pAVK	Peripheral Artery Disease
PCR	Polymerase Chain Reaction
PEI	Paul-Ehrlich-Institut
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome – Coronavirus 2
SAP	Statistical Analysis Plan

TLR	Toll-like Receptor
TNF	Tumour Necrosis Factor
ULN	Upper Limit of Normal

1 Introduction

This SAP is based on the study protocol Version 1.4 as of 8 March 2021 (EudraCT Nr. 2020-002502-75). Text citation directly from the study protocol is given in italic letters.

1.1 Background

The novel coronavirus SARS-CoV-2 causes the COVID-19 disease, … and has spread to a worldwide pandemic. T cells play the central role in SARS-CoV-2 infection and COVID-19 disease. The main goal of this study is to develop a vaccine candidate that induces superior SARS-VoV-2 T-cell immunity to better combat COVID-19.

The aim of this study is to investigate the safety and immunogenicity of a peptide vaccine consisting of SARS-CoV-2 specific HLA class II peptides in volunteers without prior or current SARD-CoV-2 infection.

The trial has been conceptualized to prove safety and immunogenicity of a peptide vaccine against SARS-CoV-2. The focus in the study population is set to older participants. This is of special interest as these people are considered to be at high risk for severe disease. Vaccination will be conducted in **two** *different healthy volunteer cohorts (Part I* **and II**[1]) with healthy adults aged 18 – 55 years in Part I, and adults aged 56 – 80 years in Part II.

1.2 Purpose of the Trial

This trial will be performed *to evaluate the safety and immunogenicity of a single use of a SARS-CoV-2-derived multi-peptide vaccine* (CoVac-1) *in combination with the TLR1/2 ligand XS15 in adults.*

2 Study Objectives

2.1 Primary Objective

The primary objective of this trial is to evaluate the safety and tolerability of the CoVac-1 vaccine, a single dose SARS-CoV-2 specific multi-peptide vaccine combined with the TLR1/2 ligand XS15 emulsified in Montanide ISA 51 VG in adults.

2.2 Secondary Objectives

Secondary objectives of this trial are to evaluate the efficacy of the CoVac-1 vaccine in terms of induction of SARS-CoV-2 specific T-cells.

2.3 Explorative Objectives

Explorative objectives are the duration and characteristics of T-cell responses and the analysis of induction of antibody responses to single SARS-CoV-2 t-cell epitopes included in the CoVac-1 vaccine.

[1] Original text in the protocol: "*Vaccination will be conducted in three different healthy volunteer cohorts (Part I - III*), *each followed by an interim safety analysis before proceeding: • Part I: Healthy adults aged 18 – 55 years • Part II: Adults aged 56 – 80… .* This text was modified in the SAP by the authors accordingly (changes in **bold**).

3 General Study Design and Plan

3.1 Overall Trial Design

This is an interventional, open-label, phase I trial evaluating the CoVac-1 vaccine, a single dose SARS-CoV-2 specific multi-peptide vaccine combined with the TLR1/2 ligand XS15 emulsified in Montanide ISA 51 VG in adults. The study is divided into two parts, which will recruit consecutively. Prior to initiation of the next part, the previous part must have completed recruiting, and day 28 of the last patient enrolled must have passed. After interim safety analysis and approval from the authorities, the next study part starts recruiting (Figure 1).

The first volunteer included in the trial will be hospitalized after vaccination and closely monitored. This patient is observed until day 28 and possibly arising safety issues are reported to and decided on by the Sponsor. Thereafter, no more than one subject per day will be treated/vaccinated. 28 days following vaccination of the 12th volunteer, there will be an interim analysis of safety and a safety review by the data safety monitoring board (DSMB) as well as a substantial amendment to the regulatory authorities (PEI and EC) before proceeding to Part II. Part II must not start recruiting prior to approval by authorities. Volunteers of part II are treated simultaneously.

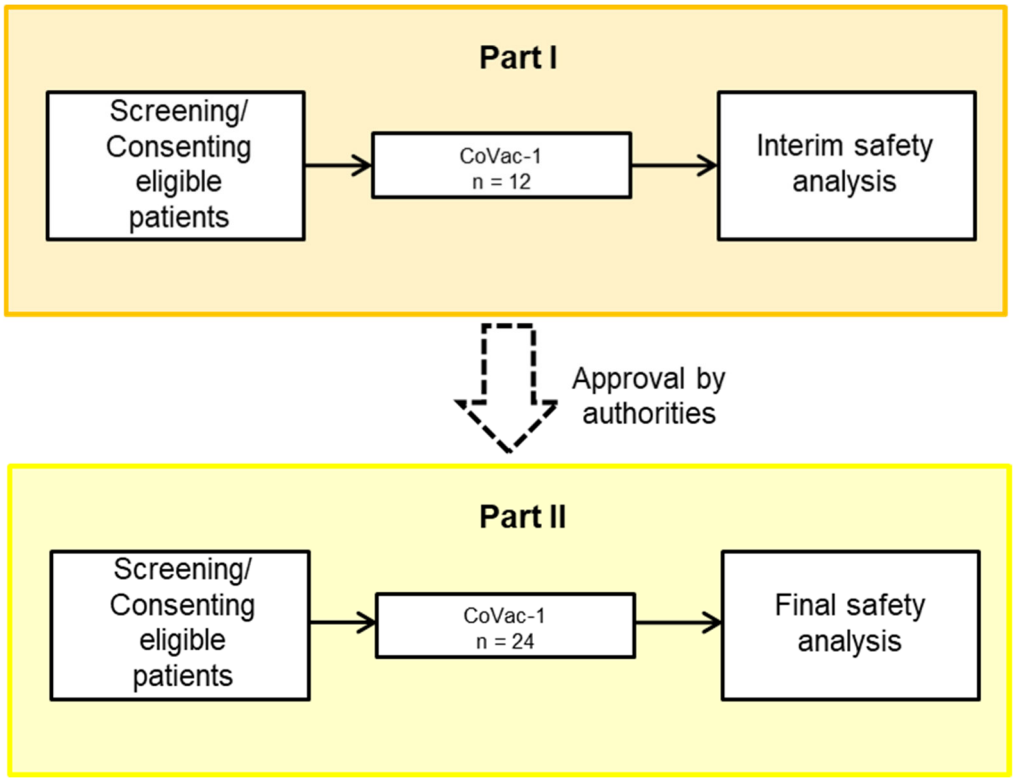

Figure 1 ***Overall Study design***

3.2 Inclusion-Exclusion Criteria

3.2.1 Inclusion Criteria

Subjects meeting all of the following criteria will be considered for admission to the trial:

1. *Adult male or non-pregnant, non-lactating female.*
    1. *Part I: Age 18 - 55 at the time of screening*
    2. *Part II: Age 56 - 80 years at the time of screening*
2. *Pre-existing medical condition*
    1. *Part I: Free of clinically significant health problems, as determined by pertinent medical history and clinical examination at study screening*
    2. *Part II: With or without pre-existing medical condition, not requiring change in therapy or hospitalization before enrollment*
3. *Ability to understand and voluntary sign the informed consent form.*
4. *Ability to adhere to the study visit schedule and other protocol requirements.*
5. *FCBP and male volunteers with partners of childbearing potential, who are sexually active must agree to the use of two effective forms (at least one highly effective method) of contraception. This should be started from the signing of the informed consent and continue until three months after vaccination.*
6. *Postmenopausal or evidence of non-childbearing status. For women of childbearing potential: negative urine or serum pregnancy test within 7 days prior to study treatment. Postmenopausal or evidence of non-childbearing status is defined as:*
    1. *Amenorrhea for 1 year or more following cessation of exogenous hormonal treatments.*
    2. *Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) levels in the post-menopausal range for women under 50.*
7. *Be willing to minimize blood and body fluid exposure of others for 7 days after vaccination*
    1. *Use of effective barrier prophylaxis, such as latex condoms, during sexual intercourse.*
    2. *Avoiding the sharing of needles, razors, or toothbrushes.*
    3. *Avoiding open-mouth kissing.*
    4. *Refrain from blood donation during the course of the study.*

3.2.2 Exclusion Criteria

Subjects presenting with any of the following criteria will not be included in the trial:

1. *Pregnant or lactating females.*
2. *Participation in any clinical study with intake of any investigational drug interfering with the study primary endpoint.*

3. *Any concomitant disease affecting the effect of the therapeutic vaccine or interfering with the study primary endpoint.*
4. *Any immunosuppressive treatment except low dose corticosteroids (≤ 10 mg prednisolone/day).*
5. *Prior or current infection with SARS-CoV-2 tested serologically or by throat/nose swab (PCR).*
6. *History of Guillain-Barré Syndrome.*
7. *Positive serological HIV, hepatitis B or C test. In case of positive HBsAg, volunteer must provide prove of hepatitis B vaccination, otherwise volunteer must be excluded.*
8. *History of relevant CNS pathology or current relevant CNS (e.g. seizure, paresis, aphasia, cerebrovascular ischemia/haemorrhage, severe brain injuries, dementia, Parkinson's disease, cerebellar disease organic brain syndrome, psychosis, coordination or movement disorder, excluding febrile seizures as child).*
9. *Baseline laboratory with lymphocytes count ≤ 1000/µl.*
10. *Only Part I: acute or chronic, clinically significant psychiatric, hematologic, pulmonary, cardiovascular, or hepatic or renal functional abnormality as determined by the Investigator based on medical history, physical exam, and/or laboratory screening test.*
11. *All parts of the clinical trial*
    - *Diabetes mellitus type II requiring drug treatment*
    - *Chronic lung disease requiring drug treatment*
    - *Any chronic liver disease or unknown abnormalities defined as:*
      - *ALT and AST ≤ 2.5 x ULN*
      - *γ-GT ≤ 2.5 x ULN*
    - *Chronic renal failure defined as GFR < 60 ml/min/1.73 m^2*
    - *Serious pre-existing cardiovascular disease such as NYHA ≥ I, coronary heart disease requiring coronary surgery or known pAVK ≥ grade 2.*
    - *Sickle cell anaemia*
    - *Obesity (as defined by age adjusted body mass index).*
12. *Hospitalization at study inclusion.*
13. *Administration of immunoglobulins and/or blood products within 120 days preceding study entry or planned administration during the study period.*
14. *History of blood donation within 30 days of enrolment or planned donations within the study period.*
15. *Known hypersensitivity to any of the components included in the CoVac-1 vaccine.*
16. *Pre-existing auto-immune disease except for Hashimoto thyroiditis and mild (not requiring immunosuppressive treatment) psoriasis.*

3.3 Method of Treatment Assignment

After screening and enrolment, volunteers will be assigned to treatment with CoVac-1.

3.4 Study Drug Administration

The CoVac-1 vaccine (500 µl) will be administered subcutaneously. A subcutaneous injection of 500 µl (approx. 250 µg per peptide, 50µg XS15) will be applied. A single vaccination per patient will be conducted.

Peptide vaccines should be injected on day 1 *into the skin at the lower part of the abdomen of the patients. The site of vaccination (right or left) will be determined by the investigator and documented.*

3.5 Study Procedures

The time points and trial procedures of this study are listed in table 1.

Table 1 Table of Events

Protocol activities and forms to be completed	***Screening***	***Vaccination phase[1]***					***Follow-up period[2]***
					Interim Safety	***EOSf***	
	≤- 7 days	***Day 1***	***Day 7 +/- 1 days***	***Day 14 +/- 1 days***	***Day 28 +/- 2 days***	***Day 56 +/- 2 days***	***3 and 6 months after peptide vaccination***
Visit		***V1***	***V2***	***V3***	***V4***	***V5***	***V6-7***
Informed consent[3]	X						
Demographics[4]	X						
Medical history[5]	X						X
Signs/symptoms[6]		X	X	X	X	X	
Enrolment[7]	X						
	Clinical assessments						
Vital signs[8]	X	X	X	X	X		
Physical examination[9]	X	X	X	X	X		
Assessment of concomitant medications[10]	X	X	X	X	X	X	
AE assessments[11]		X	X	X	X	X	X
	Laboratory assessments						
Hematology (local lab)[12]	X	X	X	X	X	X	
Blood chemistry and coagulation (local lab)[13]	X	X	X	X	X	X	
Immunoglobulins/Immuno phenotype[14]	X						
Urine analysis (local lab)[15]	X						
HBV, HCV, HIV-1, (local lab)[16]	X						
Pregnancy test[17]	X						
SARS-CoV-2 testing	X[18]						

Continued Table 1 Table of Events

Protocol activities and forms to be completed	***Screening***	***Vaccination phase[1]***					***Follow-up period[2]***
					Interim Safety	***EOSf***	
	≤- 7 days	***Day 1***	***Day 7 +/- 1 days***	***Day 14 +/- 1 days***	***Day 28 +/- 2 days***	***Day 56 +/- 2 days***	***3 and 6 months after peptide vaccination***
Visit		***V1***	***V2***	***V3***	***V4***	***V5***	***V6-7***
	Treatment						
Vaccine CoVac-1[19]		*X*					
	Efficacy assessment						
T-cell response[20]		*X*	*X*	*X*	*X*	*X*	*X*
Serological response[21]		*X*	*X*	*X*	*X*	*X*	*X*

Detailed information on schedule and activities are described in the footnotes.

1. *The peptide vaccination should be applied as early as possible after screening (max. 7 days) and approved eligibility of the volunteer. Vaccination phase will be 2 months and ends with the end of safety follow-up (EOSf).*
2. *Follow-up: After vaccination phase, volunteers will enter follow-up, which ends with the last visit 6 months after vaccination (V7, EOS).*
3. *Informed consent and volunteer registration: every volunteer must date and sign informed consent form to participate in this trial before starting any trial-related procedures.*
4. *Demographics: gender, year of birth, ethnicity*
5. *Medical history: The investigator has to collect information on the volunteers' medical history including prior illnesses, hospitalisations, and symptoms of a SARS-CoV-2 infection.*
6. *Signs/symptoms: vaccine-related and -unrelated signs and symptoms*
7. *Enrolment: volunteers are enrolled and registered through a screening procedure. Each volunteer will be registered under a specific Vol. ID on a subject log kept at the trial site.*
8. *Vital signs: At all visits: ECOG, temperature (in grade centigrade), blood pressure/pulse. At baseline additionally: height (in cm) and weight (in kg). At V4 and V5 additionally: weight (in kg). For detailed surveillance after vaccination, please refer to section* ***Fehler! Verweisquelle konnte nicht gefunden werden.*** *of the study protocol*
9. *Physical examination: inspection, abdominal, cardiac and lung auscultation, palpation of the abdomen and lymph node sites, neurological examination, inspection of vaccination site.*
10. *Concomitant medications should be reported in the respective CRF pages, including drugs used for treating AEs or, if applicable, chronic diseases.*
11. *AE assessments: events should be documented and recorded continuously. Volunteers have to be followed for AEs from application up to 56 days or until all drug-related toxicities have been resolved, whichever is later, or until the investigator assesses AEs as "chronic" or "stable". Each AE must be reported indicating the CTC (Version 5.0) grade. If an event stops and later restarts or CTC grading changes, all occurrences must be reported. A specific procedure for definition and reporting of SAEs is described in the protocol.*
12. *Hematology (local lab): hemoglobin (Hb), red blood cells (RBC), platelet count (PLT) white blood cells (WBC). Differential cell counts should be performed at baseline, at each visit during vaccination phase and thereafter at investigators discretion. Clinical status and laboratory parameters are to be followed using individual institutional guidelines and the best clinical judgment of the responsible physician, which can involve more frequent testing.*
13. *Blood chemistry and coagulation (local lab): Alkaline phosphatase (AP), total bilirubin, aspartate transaminase (AST/ SGOT), alanine transaminase (ALT/ SGPT), lactate dehydrogenase (LDH), and uric acid, C-reactive protein (CRP), sodium, potassium, calcium, blood urea nitrogen, creatinine, glucose: at baseline and during vaccination phase, thereafter at each visit using individual institutional guidelines and the best clinical judgment of the responsible physician, which can involve more frequent testing. Prothrombin time, aPTT, and fibrinogen will be measured at baseline and at investigator's discretion during treatment.*
14. *Immunoglobulin/immunophenotype: Assessment of IgA, IgG and IgM; lymphocyte subsets: T ($CD4^+$ and $CD8^+$) as well as B and NK cells.*
15. *Urine analysis (local lab): pH, glucose, proteins (qualitative, dipstick accepted): at baseline and at investigator's discretion during treatment*
16. *HBV, HCV and HIV-1: at baseline and thereafter at investigator's discretion*
17. *Pregnancy testing: For all FCBP, pregnancy testing has to be performed at the screening visit. Negative results must be available prior to vaccination.*

18. *SARS-CoV-2 testing: Volunteer must be tested for prior or current SARS-CoV-2 infection. Patients should be tested by serological test and throat/nose swab. If screening takes more than 48 hours, throat/nose swab for SARS-CoV-2 infection must be repeated. The vaccine can only be applied if a negative SARS-CoV-2 PCR test is available on the day of vaccination not older than 48 hours.If patients develop SARS-CoV-2 typical symptoms until vaccination, testing should be repeated.*
19. *Vaccine CoVac-1: Peptide vaccination should be started as soon as possible after the screening visit. Peptide vaccination will be performed once.*
20. *T-cell response: 60 ml of heparin blood for immunomonitoring and analysis of peptide specific T-cell response will be analyzed by the Walz lab, KKE Translational Immunology at the Department of Immunology, Tuebingen (central laboratory). Blood will be taken before peptide vaccination on V1, and during vaccination phase and follow-up at each visit.*
21. *Serological response: 10 ml of serum for analysis of serological response will be analysed by the Immunopathological Laboratory, University Hospital Tuebingen (central laboratory). Blood will be taken before peptide vaccination on V1, and during vaccination phase and follow-up at each visit.*

3.6 Endpoints

3.6.1 Primary Endpoint

The primary endpoint will be *the nature, frequency, and severity of AEs and/or SAEs associated with administration of CoVac-1:*

- *Solicited: ADRs/AE occurring from the time of each injection throughout 28 days following the procedure, facilitated by use of a volunteer diary.*
- *Unsolicited: AEs from the time of injection throughout 56 days following injection.*
- *Incidence of AESIs until the final study visit for each subject.*

3.6.2 Secondary Endpoints

The secondary endpoints will be *the development of a CoVac-1 specific T-cell response to at least one of the single SARS-CoV-2 T-cell epitopes included in the CoVac-1 vaccine on visit 2, 3, 4, 5 measured by IFN-γ ELISpot ex vivo and after in vitro T-cell amplification (compared to visit 1). This includes cellular conversion rate (CCR) at visit 2, 3, 4, 5 after immunization.*

3.6.3 Exploratory Endpoints

As exploratory endpoints the following parameters will be analyzed.

- *characteristic of T-cell response on visit 2, 3, 4, 5 measured by ELISpot/ICS, this includes:*
  - *Phenotyping of SARS-CoV-2 specific T-cells (CD4, CD8 etc.) by flow cytometry*
  - *Characterization of cytokine profiles of SARS-CoV-2 specific T-cells (TNF, IFN, IL-2, CD107a etc.) by intracellular cytokine staining*
  - *Recognition rate defined as percentage of peptides including a T-cell response in one individual*
  - *Intensity of T-cell response to a single SARS-CoV-2 T-cell epitope included in the CoVac-1 vaccine.*
- *Induction of long-term SARS-CoV-2 specific T-cell response 3 and 6 months after peptide vaccination.*
- *Induction of antibodies specific to the SARS-CoV-2 T-cell epitopes included in the CoVac-1 vaccine measured by ELISA. In case of unexpected detection of CoVac-1 specific antibodies the following assays will be performed:*
  - *Individual neutralization antibody titers*
  - *Seroconversion rates*
  - *Calculation of genomic mean titers (GMT) for neutralization and binding antibodies.*
- *Biomarkers and clinical characteristics influencing immunogenicity.*

4 Sample Size and Power Consideration

In this phase I study the safety/toxicity of one vaccination will be investigated. For this purpose, it will be investigated whether the incidence of severe adverse events (SAE) associated with administration of CoVac-1 exceeds a predetermined rate of 5% (= P_1 = alternative hypothesis) in the whole study population. Safety of the CoVac-1 vaccine is shown if no SAE (= P_0 = null hypothesis) occurs in the study population. An evaluable sample size of 33 achieves 81.6% power to detect a difference (P_1-P_0) of 0.0499 using a one-sided exact test based on the binomial distribution with a target significance level of 0.05. The actual significance level achieved by this test is 0.003. These results assume that the population proportion under the null hypotheses (P_0) is 0.0001. Assuming a dropout rate of 7.5% (percentage of subjects that are expected to be lost at random during the course of the study and for whom no response data concerning existence of SAE will be collected, i.e. will be treated as "missing") the total number of 36 subjects should be enrolled in the study in order to end up with 33 evaluable subjects. Sample size computed using PASS 2020 (NCSS, LLC, Kaysville, Utah, USA).

5 Data Collection and Storage

The Clinical Data Management System [secuTrial "SecuTrial"] will be used for data capture, processing and storage of study data. Data entry is performed at the investigational site by clinical staff after having received training and a user manual for the electronic CRF. The Principal investigator is responsible for ensuring that all sections of the eCRF are completed correctly and that entries can be verified against source data.

6 General Considerations

6.1 Timing of Analyses

The final analysis will be performed after the first data base look which will be performed after regularly termination of visit 5 of the last volunteer and completed data monitoring. The data base lock will be confirmed by the sponsor, and therefore later changes of data until visit 5 are not allowed.

For the follow-up period a second data base lock will be performed after regularly termination of visit 7 of the last volunteer and completed data monitoring. The data base lock will be confirmed by the sponsor.

Both data base locks will be performed after the finalization and approval of this SAP document.

6.2 Analysis Population

The analysis population consists of all included volunteers with the exception of volunteers who withdraw their informed consent for participating furthermore and for analysis of their data during the study.

6.3 Missing Data and Drop Outs

Missing values will be predicted based on plausible assumptions that account for the uncertainty due to missing data. For patients with unknown status for the primary endpoint, i.e. a volunteer without complete follow-up and without any SAE until the last known study site contact, a detailed report on the course should be presented by the investigator and discussed concerning probable unknown SAEs and the reasons for drop-out. If substantial reason will be found that the person could have experienced a SAE, this will be interpreted as failure and the recruitment should be stopped accordingly. Otherwise the safety of the person will be interpreted as success, i.e. the subject will be interpreted to have not experienced a SAE. If this decision cannot be precisely concluded, patient will be considered as drop-out. All missing data or inconsistencies will be resolved by the responsible investigator.

6.4 Results of the Interim Analysis and the Safety Interim Analysis

According to the study protocol (Version 1.4 of 08.03.2021) *the primary endpoint will be evaluated in a sequential manner after every consecutive included volunteer of part I has reached day 28. No further formal interim efficacy analysis will be performed during the conduct of the study*.

As summary of the interim safety analysis the following results were documented within the "1st Report to the Data Safety Monitoring Board for the clinical study P-pVAC-SARS-CoV-2".

Until day 28 no relevant inflammatory systemic side effect, especially no fever was reported. No allergic reactions were observed. As intended and expected all volunteers (n=12) developed a granuloma local at injection site. Further local injection site adverse events included transient erythema, itching, pain, skin ulceration and vaccination site lymphadenopathy. A summary of aforementioned AEs is reported in the tables below.

All treatment-emergent AEs

AE	***Frequency, n (%)***	***Maximum Grade***	***Frequency of maximum grade, n (%)***
Vaccination complication – erythema	*12 (100)*	*3*	*2 (16)*
Vaccination complication – swelling	*12 (100)*	*2*	*3 (25)*
Vaccination complication – induration/granuloma	*12 (100)*	*2*	*4 (33)*
Vaccination complication – itching	*10 (83)*	*1*	*10 (83)*
Vaccination complication – pain	*7 (58)*	*1*	*7 (58)*
Vaccination site lymphadenopathy	*5 (42)*	*1*	*5 (42)*
Headache	*4 (33)*	*1*	*4 (33)*
Fatigue	*3 (25)*	*1*	*3 (25)*
Nausea	*3 (25)*	*1*	*3 (25)*
Muscle cramp	*2 (16)*	*1*	*2 (16)*
Skin ulceration	*1 (8)*	*1*	*1 (8)*
Myalgia	*1 (8)*	*1*	*1 (8)*
Arthralgia	*1 (8)*	*1*	*1 (8)*
Sore throat	*1 (8)*	*1*	*1 (8)*
Dizziness	*1 (8)*	*1*	*1 (8)*
Ear pain	*1 (8)*	*1*	*1 (8)*
Diarrhea	*1 (8)*	*1*	*1 (8)*
Bloating	*1 (8)*	*1*	*1 (8)*
Sinusitis	*1 (8)*	*2*	*1 (8)*

All treatment-related AEs

AE	*Frequency, n (%)*	*Maximum Grade*	*Frequency of maximum grade, n (%)*
Vaccination complication – erythema	*12 (100)*	*3*	*2 (16)*
Vaccination complication – swelling	*12 (100)*	*2*	*3 (25)*
Vaccination complication – induration/granuloma	*12 (100)*	*2*	*4 (33)*
Vaccination complication – itching	*10 (83)*	*1*	*10 (83)*
Vaccination complication – pain	*7 (58)*	*1*	*7 (58)*
Vaccination site lymphadenopathy	*5 (42)*	*1*	*5 (42)*
Fatigue	*3 (25)*	*1*	*3 (25)*
Headache	*2 (16)*	*1*	*2 (16)*
Nausea	*2 (16)*	*1*	*2 (16)*
Skin ulceration	*1 (8)*	*1*	*1 (8)*
Myalgia	*1 (8)*	*1*	*1 (8)*
Arthralgia	*1 (8)*	*1*	*1 (8)*

Immunogenicity

Immunogenicity, in term of induction of T-cell responses to one or more of the six HLA-DR SARS-CoV-2 T cell epitopes included in the CoVac-1 vaccine was assessed before vaccination as well as on day 7, 15 and 28 after vaccination for all subjects in Part I of the study (Figure 1).

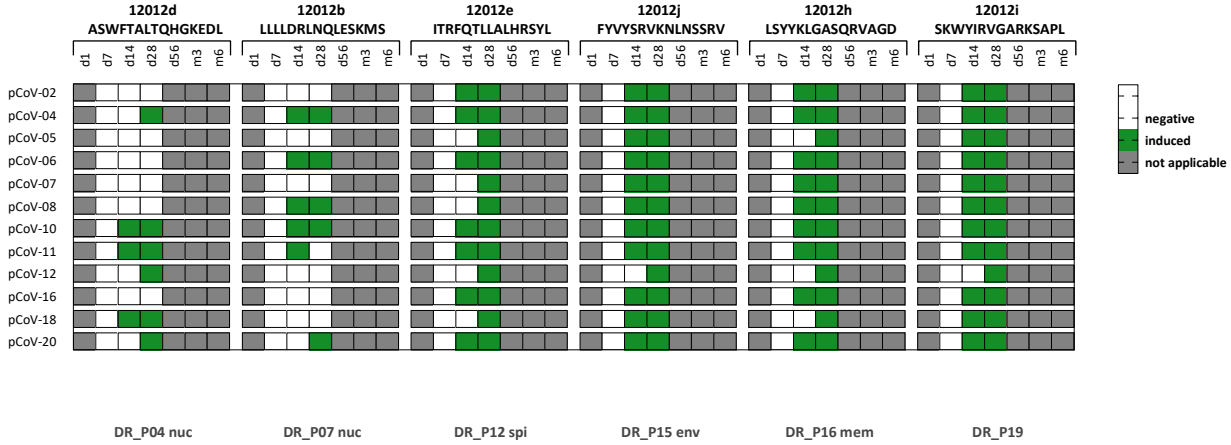

Figure 1: Induced T cell responses in the 12 subjects of the P-pVAC-SARS-CoV-2 trial assessed by ex vivo ELISPOT Induction (marked in green) of peptide specific T cell response at V2 (d7), V3 (d14), V4 (d28), as measured by ex vivo IFNγ ELISPOT is defined as positive assay (mean spot count per well is at least 3-fold higher than the mean number of spots in the negative control wells) and the spot count is at least 2-fold higher than the baseline assay (V1).

Induction of SARS-CoV-2 T cells was shown in 100 % (12/12) of subjects in part I of the study. Earliest T cell responses were observed at day 14 for 11/12 subjects. Immune responses were induced to multiple of the vaccine peptides (median 5/subject, range 4-6).

Thus, high immunogenicity of CoVAC-1 to induce early and multi-peptide T cell responses was shown.

Holding rules

To date (date of safety interim analysis)*, no adverse drug reactions or SAEs, fulfilling the criterion of the holding rules* (given in the study protocol, Version 1.4) *have been observed in the twelve study subjects.*

7 Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum, and quartiles. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for each part in the order (part I, part II, all) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

7.1 Subject Disposition

The subject disposition during the study will be shown in a CONSORT 2010 flow diagram with information to the number of volunteers enrolled (assessed for eligibility), with follow-up, and analyzed. This flow diagram will be established with 2 arms, for part I and part II separately. The number of volunteers reaching the various stages of the trial as well as the number of drop outs and for what reason will be given. Additionally, for all included volunteers the time participating in the trial will be shown. Flow charts will be established for patient numbers until V5, and for patient numbers until V7. The summary statistics will be in accordance with section 7.

7.2 Derived Variables

Within the analysis of this trial the following derived variables will be performed:

- Study duration: time from V1 to V4
- Study duration: time from V1 to V5
- Study duration with follow up: time form V1 to V7

7.3 Protocol Deviation

Protocol deviations will be categorized as major or minor prior to the database lock. Major protocol deviations are defined as follows:

- Missing visit V1, V2, V3, V4, and V5
- No vaccination or vaccination within > 7 days after screening
- Incorrect application of CoVac-1 (not abdominal)

Minor protocol deviations are defined as follows:

- Missing of V6 or V7
- Deviation from the given visit windows of ± 1 day (V2 and V3), and ± 2 day (for V4 and V5) for the scheduled visits.
- Compliance to concomitant medication
- No blood sample at a visit
- Application of another COVID-19 vaccine until V7

7.4 Demographic and Baseline Variables

The following variable groups will be considered as demographic and baseline variables:

- Demographic characteristics (age, age class for part II (56 – 64, ≥ 65), gender, ethnicity)
- Anamnestic characteristics (BMI, BMI classified (≤ 24.9, 25.0 – 29.9, ≥ 30.0), vital signs)
- Laboratory parameters (hematology, blood parameters, immunoglobulins/immuno-phenotype, urine parameters)

These variables were recorded at screening visit (see table 1). The summary statistics will be produced in accordance with section 7.

7.5 Medical History and Medications

Within the baseline visit the following variable groups were documented for medical history and treatment:

- Medical history
- Concomitant medications

These variables were recorded at screening and for concomitant medications additionally during the study at V1 – V5 (see table 1). Data will be shown within the individual data listing.

Other concomitant medication will be documented, and in case of deviation from the study protocol -

- *immunosuppressive agents apart from ≤ 10 mg prednisolone or equivalent*
- *other vaccination during the trial*
- *non-urgent medical interventions during the trial*

are prohibited - classified as major or minor protocol deviation.

Additionally *initiation of new medications, regardless of indication must be discussed with the investigator and must be noted on the participant's record*.

7.6 Treatment Compliance

Treatment compliance with respect to the study medication doesn't matter because there is only one administration (visit 1).

8 Efficacy Analyses

8.1 Primary Endpoint

The occurrence of critical events (SAE) associated with administration of CoVac-1 should be reported to the Sponsor and documented immediately in the eCRF (within 48h). The statistical center will evaluate the occurrence of critical events using automatized alerts of the e(CRF) on a daily basis and distribute this information to the Sponsor/DSMB. If one critical event will be observed, the formal statistical stopping rule of the study is reached and no further recruitment is adequate. Otherwise the safety of the procedure will be accepted, if no out of 33 volunteers will experience a critical event.

Safety of the CoVac-1 vaccine will be statistically evaluated by means of a one-sided exact test based on the binomial distribution with a target level of 0.05 to show that no SAE observed in the study population statistically confirms that the population proportion under the null hypotheses is ≤ 0.0001 (see Chapter 4).

No further statistical tests with confirmatory aim are planned.

8.2 Secondary Endpoints

Safety

The statistical analysis of the secondary endpoint will be done in a descriptive manner. No statistical tests with confirmatory aim are planned. The toxicity and safety will be described by absolute and relative frequencies using CTCAE V5.0-scoring.

Immunological Efficacy

The rate of patients with induction of peptide-specific T-cell responses within a maximum of 56 days after vaccination will be the secondary endpoint for efficacy. T-cell responses will be assessed by:

- *IFN-γ ELISPOT*
- *Intracellular cytokine staining for TNF and IFN-γ*

The rate of patients with induction of antibody responses within a maximum of 56 days after vaccination will be the secondary endpoint for efficacy. The antibody response will be assessed by ELISA.

The measurement of T-cell response will be operationalized as given in section 3.6.2. Analysis will be performed by relative and absolute frequencies of volunteers with at least one positive of the six single SARS-CoV-2 T-cell epitopes at each visit.

Cellular conversation rate (CCR) at visit 2, 3, 4 and 5 after immunization: CCR will be calculated by dividing the number of volunteers with an immune response by the number of tested volunteers at a visit.

8.3 Exploratory Endpoints

To characterize the T-cell response and induction of antibody response to single SARS-CoV-2 epitopes included in the CoVac-1 vaccine the parameters given in section 3.6.3 will be analyzed.

8.4 Subgroup analysis

According to the study protocol *exploratory subgroup analyses are planned for each part (I and II) regarding primary and secondary endpoints*.

9 Safety Analyses

According to the study protocol (Version 1.4, 08.03.2021) the following parameters will be analyzed within the safety analysis.

(Serious) Adverse Events:

- *Vital signs: pulse, blood pressure, temperature, and weight*
- *Physical examination including inspection of the vaccination side*
- *Clinical laboratory evaluations:*
  Hematology: with blood cell (WBC), hemoglobin (Hb), platelet count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC)
  Chemistry: AP, total bilirubin, AST/SGOT, ALT/SGPT, LDH, and uric acid, CRP, sodium, potassium, calcium, blood urea nitrogen, creatinine, glucose
- *Concomitant medications*
- *(S)AEs by NCI CTCAE Version 5.0 and as in appendix 14.5* of the study protocol.

The summary statistics will be produced in accordance with section 7 for (S)AEs. Other data will be shown within the individual data listing.

10 Reporting Conventions

P-values ≥ 0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "< 0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

11 Technical Details

At the time of writing the statistical analysis plan SAS Version 9.4 is used.

For data capture secuTrial® database version 5.6.2.3 is used.

12 Summary of Changes to the Protocol

The following changes to the study protocol will be performed:

- Despite the given information that "… *vaccination will be conducted to three different healthy volunteer cohorts (Part I – III)…"* the vaccination was conducted in two different healthy volunteer cohorts (Part I and Part II) with adults aged 18 - 55 years in Part I, and adults aged 56 - 80 years in Part II (see section 1.1).

- According to section 6.1 for the final analysis of the study data two data base lock time points will be performed. First for patients with regularly termination of visit 5 of the last volunteer and completed data monitoring, and second for patients with regularly termination of visit 7 of the last volunteer and completed data monitoring.

13 Data Source

The study data were hold within a secuTrial® data base. For the final analyses of the study the following data files will be transferred 1 : 1 to the statistical analysis system SAS:

- Screening: mnpp085scr, including emnpp085othcm, emnpp085actmed
- Enrolment: mnpp085enrol
- Baseline (Vaccination): mnpp085v1b, including emnpp085actmed; mnpp085v1v, including emnpp085vsfind, emnpp085vs
- Visit 2 – 5: mnpp085v2, including emnpp085vsfind, emnpp085mednew, emnpp085medstop, emnpp085medchange
- Visit 6 – 7: mnpp085fu, including emnpp085othcm
- End of study: mnpp085es
- T-cell response: mnpp085immo
- AE: mnpp085ae, including emnpp085aec

No other data sources will be used within the final data analyses.

14 Planned Tables, Figures and Listing

Table 2 Planned tables, figures and listing for the statistical study report

Number	Title	Parameters
1	**Study Information**	
Figure 1	CONSORT 2010 flow diagram	Screened, eligible, enrolment; population divided up to the groups Part I and Part II
1.1	Observation of study entry	
1.1.1	Inclusion criteria	Inclusion criteria 1 – 8
1.1.2	Exclusion criteria	Exclusion criteria 1 – 16
1.2	Study termination	
1.2.1	Number of visits	Visits
1.2.2	Study duration per patient (distribution)	Time from visit 1 to visit 5
1.2.3	Follow-up visits per patient (distribution)	Time from visit 1 to visit 7
1.2.4	End of study or termination, drop out	Reason for end of study
2	**Descriptive Analysis** (for each parameter all visits)	
2.1	Demographic characteristics	Age, age in classes, gender, ethnicity
2.2	Anamnestic characteristics	BMI, BMI classes
2.3	Medical history	Chronic headache – other study relevant co-morbidities
2.4	Vital signs	Body temperature, heart rate, systolic and diastolic blood pressure, ECOG, body weight
2.5	Vital signs right after vaccination	Body temperature, heart rate, systolic and diastolic blood pressure, oxygen saturation each after 30, 60, 90, 120 minutes
2.6	Laboratory assessments	Hematology, Blood chemistry and coagulation, Immunoglobulins/ immunophenotype, Urine analysis
2.7	Signs and symptoms	Fever – diarrhea
2.8	Physical examination	
2.9	Vaccination	Dimetinden, location of vaccination
2.9.1	Investigation of vaccination site	Formation of granuloma – other suspicious findings at the injection site
2.9.2	Specific immune mediated diseases	Neuroinflammatory disorder – other immune mediated disease
2.9.3	Novel SARS-CoV-2 positivity/infection	Occurrence, accompanied by symptoms
2.10	Concomitant medication	Change since last assessment, new concomitant medication
2.11	T-cell response	Ex vivo ELISPOT ELISPOT after 12-d IVS
2.12	Health conditions	Chronic headache – other study relevant co-morbidities
2.13	Adverse events/serious adverse events	CTCAE term, grading, SAE, relationship, outcome

Continued Table 2 Planned tables, figures and listing for the statistical study report

Number	Title	Parameters
3	**Statistical Analysis**	
3.1	Primary endpoint (documented within the visit eCRF)	Number of solicited and unsolicited ADRs or AEs, Incidence of AESIs
3.2	Secondary endpoints	T-cell response: IFN-γ ELISpot, cellular conversion rate
3.3	Exploratory endpoints	Phenotyping Cytokine profiles Recognition rate Intensity of T-cell response Induction of long-term T-cell response Induction of specific antibodies Biomarkers Clinical characteristics
4	**Safety Analysis**	
4.1	AEs	Max. severity CTC grading, duration, frequencies, related und unrelated, outcome
4.2	SAEs	Max. severity CTC grading, duration, frequencies, related und unrelated, outcome
5	**Individual Data Listing**	
5.1	Visit 0 (Screening and Enrolment)	
5.2	Visit 1 (Baseline-Vaccination)	
5.3	Visit 2	
5.4	Visit 3	
5.5	Visit 4	
5.6	Visit 5	
5.7	Visit 6	
5.8	Visit 7	
5.9	End of study (termination)	
5.10	T-cell response	
5.11	AEs/SAEs	