Sponsor	University Hospital of Tuebingen represented by Medical Director: Prof. Dr. med. M. Bamberg Director of Administration: G. Sonntag
Title	P-pVAC-SARS-CoV-2: Phase I single center safety and immungenicity trial of multi-peptide vaccination to prevent COVID-19 infection in adults
Short Title	P-pVAC-SARS-CoV-2
Coordinating Investigator (Leiter der klinischen Prüfung, According to § 4 German Drug Law (AMG)) Co-Coordinating Investigator Sponsor's Delegate Scientific Coordinator	PD Dr. med. Juliane Walz Dr. med. Jonas Heitmann Prof. Dr. med. Helmut Salih Prof. Dr. rer. nat. Hans-Georg Rammensee / Prof. Dr. rer. nat. Stefan Stevanović
Indication	Part I: Adults aged 18-55 years Part II: Adults aged 56-74 Part III: Adults aged ≥ 75
Number of Volunteers	Total number of volunteers: 36 Part I: 12 Part II: 12 Part III: 12
Inclusion Criteria 07.10.2020	1. Adult male or non-pregnant, non-lactating female 1. Part I: Age 18-55 at the time of screening 2. Part II: Age 56-74 years at the time of screening 3. Part III: Age ≥ 75 years at the time of screening 2. Pre-existing medical condition 1. Part I and II: Free of clinically significant health problems, as determined by pertinent medical history and clinical examination at study screening

	2. 3. Ability to understand and voluntarily sign an informed consent form 4. Ability to adhere to the study visit schedule and other protocol requirements 5. Female volunteers of child bearing potential (FCBP) and male volunteers with partners of child bearing 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
Inclusion criteria	6. Postmenopausal or evidence of non-child-bearing 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. Amenorrhoea for 1 year or more following cessation of exogenous hormonal treatments 2. Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) levels in the postmenopausal range for women under 50 7. Be willing to minimize blood and body fluid exposure from 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 8. Refrain from blood donation during the course of the study
Exclusion Criteria	1. Pregnant or lactating females 2. Participation in any clinical study with intake of any investigational drug interfering with the study primary endpoint including: o Active infection

	○ Psychatric disorders ○ Known systemic anaphylaxis 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 (equivalent to ≤10mg 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 pathology (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 lymphocyte count ≤ 1000/µl 10. <u>Only Part I</u> ○ 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 Typ II requiring drug treatment ○ Chronic lung disease requiring drug treatment ○ Any chronic liver disease or unknown liver 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/1,73m^2 ○ Serious pre-existing cardiovascular disease such as NYHA ≥ I, coronary heart disease requiring coronary surgery or known pAVK ≥ grade 2 ○ Sickle cell anemia

	○ Obesity (as defined by age adjusted body mass index) 12. Hospitalization at study inclusion 13. Administration of immunoglobulins and/or any blood products within the 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
Description of the Medical Products	IMP/Drug product/Peptide vaccine: CoVac-1 applied as one multipeptide cocktails consisting of: 1. SARS-CoV-2 peptides: Six promiscuous HLA-DR-restricted peptides (240 µg each) derived from different proteins of SARS-CoV-2 2. XS15: The lipopeptide XS15 is a water-soluble synthetic Pam_3Cys-derivative. As TLR1/2 ligand it will be included as an adjuvant in the peptide vaccine. Peptides are synthesized in the GMP-certified Wirkstoffpeptidlabor at the University of Tuebingen (Prof. Stefan Stevanović) and will be formulated at the GMP-Center of the University Hospital Tuebingen. The GMP-certified Wirkstoffpeptidlabor specializes in multipeptide cocktails with variable composition and holds a production permit (Herstellungserlaubnis) for different multipeptide cocktails including the TLR 1/2 ligand XS15. 3. Montanide ISA 51 VG: Prior to application, the peptide cocktail (consisting of 6 SARS-CoV-2-derived peptides and XS15) will be emulsified in a water-oil emulsion 1:1 with Montanide ISA 51 VG to a final volume of 500 µl.
	Treatment schedule: A single vaccination with the IMP CoVac-1 (SARS-CoV-2 HLA-DR peptides, XS15 emulsified in Montanide ISA 51 VG) (500 µl) will be applied subcutaneously (s.c.) to the abdominal skin.

Study Design:	Single center Phase I clinical trial Part I: 12 subjects will receive an open-label 500 µl subcutaneous injection via needle and syringe of the study IMP (CoVac-1). No more than one subject per day will be enrolled. 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 an amendment to the regulatory authorities (Paul-Ehrlich Institute and Ethics Committee) before proceeding to Part II. Part II: 12 subjects will receive an open-label 500 µl subcutaneous injection via needle and syringe of the study IMP (CoVac-1). 28 days following vaccination of the 12th volunteer, there will be an interim analysis of safety and a safety review by the DSMB as well as a substantial amendment to the regulatory authorities (Paul-Ehrlich Institute and Ethics Committee) whether to proceed to next Part III. Part III: 12 subjects will receive an open-label 500 µl subcutaneous injection via needle and syringe of the study IMP (CoVac-1).
Aim of the Study	To evaluate the safety and immunogenicity of a single use of a SARS-CoV-2-derived multi-peptide vaccine in combination with the TLR1/2 ligand XS15 in adults

Objectives/Endpoints	Primary endpoint:
	
	• The nature, frequency, and severity of AEs and/or SAEs associated with administration of CoVac-1:
	  • Solicited: ADRs/AEs 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
	  • SAEs from the time of injection until the final study visit for each subject
	  • Incidence of AESIs until the final study visit for each subject
	
	Secondary endpoints:
	
	• 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 Visits 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 Visits 2, 3, 4, 5 after immunization
	<u>Explorative endpoints:</u> • Characteristics of T-cell response on Visits 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 inducing 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 responses 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 In case of unexpected detection of CoVac-1 specific antibodies the following assays will be performed: - Individual neutralization antibody titers - Seroconversion rates - Calculation of geometric mean titers (GMT) for neutralizing and binding antibodies • Biomarkers and clinical characteristics influencing immunogenicity.
Statistics, Safety Variables and Stopping Rules	**Safety**: 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% (= P1 = alternative hypothesis) in

	the whole study population. Safety of the CoVac-1 vaccine is shown if no SAE (= P0 = null hypothesis) occurs in the study population. An evaluable sample size of 33 achieves 81.6% power to detect a difference (P1-P0) 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 (P0) 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). **Sample size:** 36 Part I: n=12 Interim Safety Analysis after Part I and a substantial amendment to authorities Part II: n=12 Interim Safety Analysis after Part II Part III: n=12
Database	A validated GCP conform clinical trial database hosted by the IKEAB Tuebingen (SecuTrial) will be used for data capture and validation in this trial
Participating Centers and Investigators	CCU Translational Immunology, Department of Internal Medicine, University Hospital Tuebingen, (Prof. Dr. Salih, PD Dr. Walz)
Study Type	• AMG
Competent Regulatory Authorities	• PEI and EC
Monitoring according GCP	Monitoring of the clinical trial will be performed by the ZKS Tuebingen.

Study duration	Total study duration for individual volunteer: 6 months Safety duration for individual volunteer: 8 weeks Follow up (exploratory end points) for individual volunteer: 4 months
Length of Study/ Time Lines	Total trial duration: 1 years Duration for individual patient: Safety follow-up: 8 weeks Follow-up: 4 months Number of visits: 8 FSI (First Subject In): Q3/2020 LSI (Last Subject In): Q1/2021 LSO (Last Subject Out): Q3/2021 DBL (Data Base Lock): Q3/2021 Statistical Analyses Completed: Q4/2021 *Trial Report Completed: Q4/2021*