CLINICAL TRIAL PROTOCOL

naNO-COVID

A Phase I, double-blind, randomized, vehicle-controlled, dose-finding, safety study of a synthetic nanoparticle-based, T cell priming peptide vaccine against SARS-CoV-2 in healthy adults in Switzerland.

naNO-COVID: A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV-2

NOTE: This is stage 2 of a 2-stage adaptive trial investigating the safety of 2 vaccines from a single nanoparticle vaccine platform for emerging diseases

- **Stage 1:** naNO-DENGUE *= refer to approved trial 2020-02258 (naNO-DENGUE_ClinicalTrialProtocol_V3.0_English_18Dec2020)*
  A Phase-I study of a nanoparticle-based peptide vaccine against Dengue (**Master** protocol)
- **Stage 2:** naNO-COVID ***= THIS DOCUMENT***
  A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV-2 (**Sub**-protocol prospective amendment. Implemented after interim safety analysis of stage 1 and approval by the DSMC, CEC and Swissmedic)

Study Type:	Clinical trial with Investigational Medicinal Product (IMP) This is a prospective amendment (submitted as a Sub-protocol of a 2-stage adaptive trial)
Study Categorisation:	First in human (ClinO - Category C)
Study Registration:	Clinicaltrials.gov Swiss National Clinical trial Portal (SNCTP via BASEC)
Study Identifier:	naNO-COVID
Investigational Product:	**PepGNP-Covid19:** MHC-class-I binding synthetic peptides derived from the SARS-CoV-2 bound to gold nanoparticles
Protocol Version and Date:	V3.0_01.02.2022

Sponsor:

Emergex Vaccines Holding Limited

Dr Athanasios Papadopoulos

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Sponsor's representative in Switzerland:

Prof Blaise Genton

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Signature page

Study Title: naNO-COVID: A Phase I, double-blind, randomized, vehicle-controlled, dose-finding, safety study of a synthetic nanoparticle-based, T cell priming peptide vaccine against SARS-CoV-2 in healthy adults in Switzerland.

The Sponsor, Sponsor's representative in Switzerland and trial statistician have approved protocol V3.0 dated 01 February2022 and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Sponsor: Emergex Vaccines Holding Limited (representative: Dr Athanasios Papadopoulos)

Feb 1, 2022 — Athanasios Papadopoulos (Feb 1, 2022 14:38 GMT)

Place, Date — Signature

Sponsor's representative in Switzerland: Prof Blaise Genton

Place, Date — Signature

Trial Statistician: Mohamed Faouzi

Place, Date — Signature

Local principal investigator at study site:
I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Association Declaration of Helsinki, ICH-GCP and the local legally applicable requirement.

Site: Unisanté, Lausanne

Principal Investigator: Prof Blaise Genton

Place, Date — Signature

Table of Contents

ABBREVIATIONS

Ab	Antibody
ADE	Antibody Dependent Enhancement
AE	Adverse Event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
APC	Antigen presenting cells
AST	Aspartate aminotransferase
BASEC	Business Administration System for Ethical Committees, (https://submissions.swissethics.ch/en/)
bpC	Base particle Comparator (gold nanoparticles (**GNP**) without peptides)
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CH	Switzerland
CHUV	Centre Hopsitalier Universitaire Vaudois
CI	Confidence interval
ClinO	Ordinance on Clinical Trials in Human Research
CRF	Case Report Form
CTCAE	Common terminology criteria for adverse events
CTL	Cytolytic T lymphocytes
CTU	Clinical Trial Unit
PepGNP-Covid19	Investigational medicinal product of this study: an MHC class I-binding synthetic peptides derived from SARS-CoV-2 bound to gold nanoparticles NOTE: in the IB, the vaccine is referred to interchangeably as • Generically: RNA peptide (set-point) T-cell vaccine product and GNP-P • Specifically: SARSCoV2 T-vaccine and PepGNP-Covid19
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FBC	Full Blood Count
g/G	Grams/Giga
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
GNP	Gold nanoparticle
H0/H1	Null/Alternative hypothesis
HBV/HCV	Hepatitis B virus / Hepatitis C virus
hCG	Human chorionic gonadotropin (pregnancy hormone)
HD	**High dose group**

HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HRA	Federal Act on Research involving Human Beings
Ht	Haematocrit
IB	Investigator brochure
ICH	International conference on harmonization
ICS	Intracellular cytokine staining
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN-γ	Interferon gamma
IMP	Investigational Medicinal Product
IPC	Infection Prevention control
ISO	International Organisation for Standardisation
ITT	Intention to treat
IU	International Units
IUD/S	Intrauterine Device/System
l/L	Litre
LD	**Low dose group**
MCV	Mean Corpuscular Volume
mg	Milligram
MHC	Major Histocompatibility Complex
ml/ML	Millilitre
mmol	Millimole
MTA	Material Transfer Agreement
NP	Nanoparticle
nmol	Nanomole
NSAID	Non-steroidal anti-inflammatory drug
PBMC	Peripheral blood mononuclear cell
PI	Principal investigator
PIS	Participant information sheet
PRNT	Plaque reduction neutralization test
PT	Prothrombin time
PV CRO	Pharmacovigilance Contract Research Organisation
RBC	Red Blood Cell
S-Protein	Spike protein of the SARS-CoV-2 virus
SAE	Serious adverse event
SARS-CoV-2	**Severe Acute Respiratory Syndrome Coronavirus-2**
SOP	Standard Operating Procedure
SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial Master File

ULN	Upper limit of normal
µmol/µl	Micromole/microliter
VoC	Variant of Concern
vehicle-GNP	Base-Particle Comparator (comprised of a gold nanoparticle)
WFI	Water for injection
WHO	World Health Organization

STUDY SYNOPSIS

Sponsor	Emergex Vaccines Limited
Study Title	**naNO-COVID:** A Phase I, double-blind, randomized, vehicle-controlled, dose-finding, safety study of a synthetic nanoparticle-based, T cell priming peptide vaccine against SARS-CoV-2 in healthy adults in Switzerland
Short Title / Study ID	**naNO-COVID:** A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV-2
Protocol Version	V3.0, 01.02.2022
Trial registration	• ClinicalTrials.gov registry • Swiss National Clinical trial Portal (SNCTP via BASEC)
Study category and Rationale	Category C as per ClinO First in humans Phase I vaccine
Clinical Phase	Phase I
Submission type	**Adaptive trial** This is the second stage (prospective amendment sub-protocol) for a 2-stage study investigating the safety of 2 vaccines from a nanoparticle vaccine platform for emerging diseases: • **Stage 1:** naNO-DENGUE *= refer to approved trial 2020-02258 (naNO-DENGUE_ClinicalTrialProtocol_V3.0_English_18Dec2020)* A Phase-I study of a nanoparticle-based peptide vaccine against Dengue virus (**Master** protocol) • **Stage 2:** naNO-COVID ***= THIS DOCUMENT*** A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV2 (**Sub**-protocol, implemented after interim safety analyses of stage 1 and approval by DSMC, CEC and Swissmedic) **Thus, this is a sub-protocol submitted as a prospective amendment** See **Figure 2** for an overview
Hypotheses	1) The scale of the COVID-19 pandemic requires multiple vaccine candidates to ensure democratic and rapid access to protection by: o Providing a range of vaccine choices tailored to variations in immunological profiles across demographics as well as suited to environments with various levels of resources (cold chain etc). o Distributing and parallelizing manufacture, to speed up scale, avoid reagent stockouts and dilute monopolies 2) The ability for SARS-CoV-2 to mutate, requires multiple vaccine candidates to ensure robust and sustainable protection. Vaccines with a range of epitopes and immune targets provide immunological diversity and reduce vulnerability to mutant escape. 3) Nanotechnology fulfils the needs of a COVID vaccine by being a rapidly scalable and modular platform 4) Humoral immunity may be transient and insufficient against emerging variants of SARS-CoV-2 5) Cellular immunity against SARS-CoV-2 is lasting and associated with recovery in COVID-19

Background and Rationale	**The rationale for a(-nother) COVID-19 vaccine** Despite drastic quarantine measures, SARS-CoV-2 continues to propagate and threaten global economies and healthcare systems. There is universal consensus on the need for vaccination to protect against complications, reduce viral shedding and therefore prevent transmission. Sixty- three days after the SARS- CoV- 2 sequence was published, the first dose of the first experimental vaccine was tested in humans. Fifteen months later, 183 vaccines are in development in 101 clinical trials. At the time of writing, some COVID vaccines have been approved by the regulatory authorities across the globe and at least three have reported approximately 95% efficacy to infection and almost complete protection against severe disease against the first variant of SARS-COV-2. The list of coronavirus Variants Of Concern (VOC) has grown, and further, faster transmitting variants have emerged around the globe, such as the current delta variant. Coronavirus harbours the potential to become a seasonal disease. It is moving towards being a “new flu”, with potential perennial circulation and continuously evolving VOCs, needing diverse vaccines to control it. The global vaccine alliance, GAVI, has specifically advocated for vaccine diversity as a means to ensure equitable efficacy and access as well as a means of maximizing scalability. Having multiple vaccines improves the robustness and sustainability of global protection by: • Creating immunological diversity to protect against mutant escape, the risk of which is expected to increase with growing selective pressure in an increasingly immunized population. • Tailoring vaccines to individual needs including vulnerable populations, immunological reactivity and allergic risk profiles • Diversifying manufacturing methods, and thus reducing risk of global reagent stockouts and largescale contaminations. Parallelizing production also ensures greater availability sooner. • Democratizing access with competitive pricing, diluting monopoly and representing needs in resource-constrained environments.

	The proposed vaccine of this trial also specifically seeks to diversify the immunological target response by carefully curating an MHC-I binding antigen cocktail from the SARS viruses ligandome and loading it onto a robust nanoparticle for controlled release and coupled T cell immunostimulation. **Rationale for targeted cellular immunity** • ***Humoral immunity may be transient and insufficient*.** While early vaccine efforts focused on antibody responses, evidence is now mounting for the importance of cellular immunity in acute infection and in the maintenance of immunological memory. Evidence indicates that a robust antibody (Ab) response alone may be insufficient to avoid severe disease and might even promote it in some circumstances. It then becomes important to diversity the immunological profile of vaccine strategies to cover the possibility of an Ab-escape VOC. • ***Cellular immunity is lasting and associated with recovery.*** T cell memory in respiratory coronaviruses was recorded to be long-lived (>6–17 years) and targeted against epitopes that were less prone to mutation and escape(1). Indeed, studies have shown there to be a particularly, robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. In SARS, cytotoxic CD8+ T cells were required for virus clearance and in COVID-19, several clinical studies have identified reduced CD8+ T cells as an early prognostic indicator of severe or lethal disease and treatment efficacy, prompting calls that “an effective COVID-19 vaccine needs to engage T Cells” This vaccine selects peptides from the SARS virus ligandome that specifically and exclusively engage the MHC-I receptor across a range of HLA haplotypes in an effort to elicit a lasting cellular immune response. It also specifically seeks to avoid generating a humoral response, which has the potential to be transient and even detrimental in certain cases.
	The rationale for a nanoparticle delivery system • ***Dose-sparing.*** Nanoparticle antigen delivery systems are designed to protect antigens from premature proteolytic degradation and control their release as well as facilitating antigen uptake and processing by antigen presenting cells. Increasing the effective activity-per-unit, allows the reduction of effective antigen dose (to nanomoles). Such dose-sparing strategies become critical in scaling production, which is why NP platforms are considered to be highly promising for COVID-19 control. • ***Immunomodulation.*** A more classic dose-sparing strategy in vaccine production, is the addition of an adjuvant. Interestingly, NPs have shown intrinsic immunomodulatory functions, acting as adjuvants or immune potentiators. • ***Targeted, controlled release.*** NPs not only improve the immunogenicity and stability of antigen, but also achieve targeted delivery and sustained release. Thus, avoiding the high-dose “antigen dump” of traditional vaccines as well as co-localising adjuvant and antigen, to avoid non-specific stimulation. • ***Rapidly scalable to new epitopes.*** Many view the greatest advantage of the NP platform is that it may serve as a “plug-and-play” technology that can be tailored to seasonal or new strains of coronaviruses.
Study design	Double-blind, vehicle-controlled, randomised trial
	This is stage 2 of a 2-stage study investigating the safety of a peptide T cell inducing vaccine platform for emerging diseases • **Stage 1: naNO-DENGUE:** A Phase-I study of a nanoparticle-based peptide vaccine against Dengue • **Stage 2: naNO-COVID:** A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV2 This is a prospective major amendment in the form of a subprotocol and is submitted along with preliminary safety data from stage 1.

Objective(s)	**Primary:** To evaluate the safety and reactogenicity of two intradermal injections of two different doses of the investigational COVID peptide T cell inducing vaccine (**PepGNP-Covid19**) administered to healthy volunteers in Switzerland as a: 1) candidate vaccine for the prevention of COVID-19 2) proof-of-concept for a rapidly scalable modular peptide vaccine platform
	Secondary: 1) To assess the evidence of a CD8 T-cell mediated immune response as a surrogate of protection against severe COVID-19 using a novel peptide set point vaccine in healthy adults. 2) To assess the presence of an antibody mediated response
Outcome(s) *See* **Table 1**	**Primary: Assess the safety, tolerability and reactogenicity of the candidate vaccine (PepGNP-Covid19)** • Occurrence of solicited local reactogenicity signs and symptoms [Time Frame: 7 days following each vaccination] • Occurrence of solicited systemic reactogenicity signs and symptoms [Time Frame: 14 days following each vaccination] • Occurrence of unsolicited adverse events [Time Frame: 6 months following enrolment. *i.e. entire trial period*] • Occurrence of serious adverse events (SAEs) [Time Frame: 6 months following enrolment. *i.e. entire trial period*] • Occurrence of adverse events of special interest (**Section 10.1.1.3)** [Time Frame: 6 months following enrolment *i.e. entire trial period*] • Change from baseline for safety laboratory measures [Time Frame: 6 months following enrolment *i.e. entire trial period*] *Note: all AEs will be recorded throughout the study according to Swiss ClinO ordinance. The timelines above refer only to outcome measures.*
	Secondary: 1) **Assess cellular immunogenicity of the candidate vaccine (PepGNP-Covid19):** • Change from baseline of the frequency CD8+ T cell specific to PepGNP-Covid19, proportion of participants with a positive response and characterisation of the memory phenotype of the response. [Time Frame: 6 months following enrolment] 2) **Assess the humoral immunogenicity of the candidate vaccine (PepGNP-Covid19):** • (For individuals seronegative at enrolment) Proportion of participants becoming seropositive (antibodies against SARS-CoV-2) • (For individuals seropositive at enrolment) Fold change in anti-SARS-CoV-2 antibody titres • [Time Frame: 6 months following enrolment *i.e. entire trial period*]

Table 1. *Monitoring periods for safety outcomes*

Day of study	**0**	**7**	**14**	**21**	**28**	**35**	**180**
	1st injection			*2nd injection*			
Solicited local reactogenicity	7 days			7 days			
Solicited systemic reactogenicity	14 days			14 days			
Unsolicited adverse events	Duration of study						
SAE	Duration of study						
Safety laboratory measures	Duration of study						
Cellular immunogenicity	Duration of study						
Humoral immunogenicity	Duration of study						
All adverse events recorded	Duration of study						

Inclusion criteria	An individual must fulfil <u>all</u> of the following criteria in order to be eligible for trial enrolment: 1. Aged 18 to 45 years on the day of inclusion 2. Participant signed informed consent 3. Residing in Switzerland
Exclusion Criteria	**An individual fulfilling <u>any</u> of the following criteria is to be excluded from enrolment:** 1. Participant is pregnant, lactating, or of childbearing potential[1] 2. Participation in the 4 weeks preceding the first trial vaccination or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure 3. Receipt of any vaccine (including vaccination against COVID) in the 4 weeks preceding the first trial vaccination (excepting influenza vaccination, which may be received up to 2 weeks before first study vaccine) or planned receipt of any vaccine in the 4 weeks following each trial vaccination. 4. Documented COVID-19 disease in the 4 weeks preceding the first trial injection. 5. Receipt of immunoglobulins, blood or blood-derived products in the past 3 months 6. Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy[2] 7. Self-reported or documented seropositivity for human immunodeficiency virus (HIV), hepatitis B natural infection (HBcAb positive serology), or hepatitis C 8. Known systemic hypersensitivity to any of the vaccine components (e.g. gold), or history of a life-threatening reaction to vaccines 9. Current alcohol abuse or drug addiction (reported or suspected) 10. Chronic illness that, in the opinion of the investigator, is at a stage where it might interfere with trial conduct or completion (i.e. any risk factor for a severe COVID-19 disease including BMI > 30 kg/m2) 11. Thrombocytopenia or any coagulation disorder 12. Identified as an Investigator or employee of the Investigator or study centre with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study (i.e. in the employment of the Tropivac clinic or DFRI unit at Unisanté). 13. Refusal to be informed in the event that relevant results concerning the participant's health are revealed
Exclusion Criteria at the Time of Vaccination *(where delayed administration is possible)*	The following events constitute contraindications to the administration of the investigational product on the day of planned vaccination. The participant must be followed until resolution of the event as with any medical event and may be considered for vaccination at a later date (maximum 14 days later) or withdrawn at the discretion of the Investigator. **Delays due to these events do not constitute a protocol deviation.** • Temperature of >37.5°C at the time of vaccination • Acute disease[3] at the time of vaccination • If there is a clinical suspicion of COVID-19 (according to the clinician's judgement), the clinical team will need to wait for the result of the PCR test for SARS-CoV2, even if the rapid test is negative, and the vaccination will be delayed until the result comes back negative, the symptoms have resolved, and at least 4 weeks have elapsed since the first positive test result.

[1] An individual who does **<u>not</u>** have childbearing potential is defined as a female who is:
- Pre-menarche or post-menopausal for at least 1 year
- Surgically sterile
- Using an effective method of contraception from at least 4 weeks prior to the first vaccination until at least 10 weeks after the last vaccination (up to day 90). Effective contraception methods are described in the appropriate section.
- Has no heterosexual intercourses

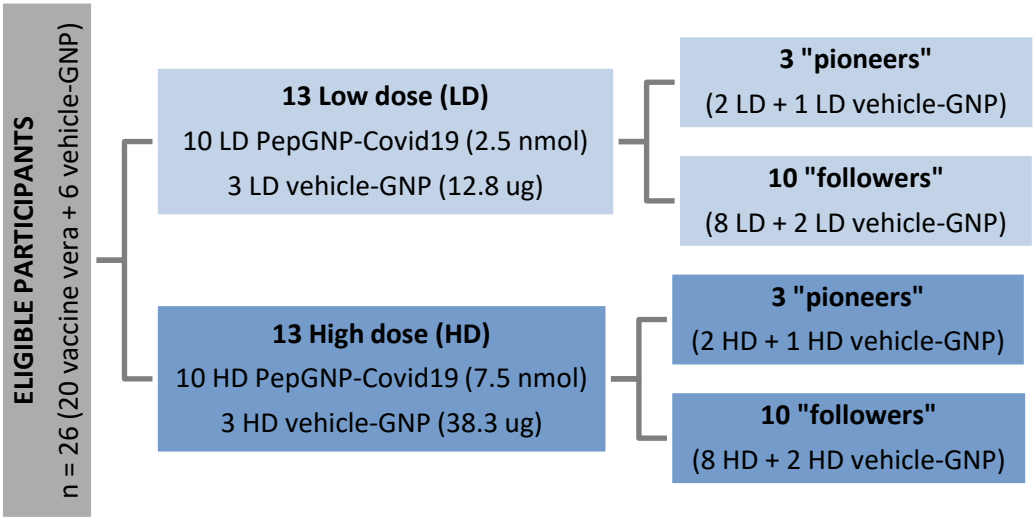
[2] Such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)

[3] "Acute disease" is defined as the presence of a moderate or severe illness with or without fever according to investigator's judgment. All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. axillary temperature of ≤37.5°C.

Measurements and procedures *See **Figure 2** for full overview*	**Recruitment:** This study includes healthy adults aged 18-45 years residing in Switzerland. **Information and consent:** All individuals showing interest in participating in the study will be provided with an information sheet and given a minimum of 72 hours to decide whether they would like further information from the study investigator and/or sign the consent if willing. **Screening:** Consenting participants will then schedule a screening appointment where they undergo eligibility testing comprising a structured interview on their medical history as well as a targeted physical exam. Blood and urine samples will also be collected for clinical laboratory tests which include general assessments of organ function[4] as well as screening for a panel of infectious diseases [5]. Specifically, all eligible participants will be screened for SARS-CoV-2 exposure. All eligible female participants will undergo a human chorionic gonadotropin β-subunit (βhCG) urine pregnancy test before receiving any vaccination (performed again on the day of both proposed vaccinations to ensure unchanged pregnancy status). **Randomisation:** 26 eligible participants will be randomized in the following groups: **Group 1** (n=13) • 10 Low Dose (LD) PepGNP-Covid19 (2.5 nmol peptide + 12.8ug GNP) • 3 LD Base Particle Comparator (12.8ug GNP) **Group 2** (n=13) • 10 High Dose (HD) PepGNP-Covid19 (7.5 nmol peptide + 38.3ug GNP) • 3 HD Base Particle Comparator (38.3ug GNP) Thus, 20/26 vaccine vera and 6/26 Base Particle Comparator (bpC). Allocations of vaccine vera vs bpC for each group are double-blinded. Enrolment will follow a dose escalation strategy (LD--> HD) conditional to a Go/No Go by DSMC review. **Intervention:** Two intradermal injections of the IMP or control will be administered in the upper arm of each participant. The injections will take place on d0 and d21 using the Nanopass MicronJet600 (https://www.nanopass.com/product/) microneedle. The participant will be monitored for 60min after each vaccination for immediate adverse reactions. Before any vaccination, a baseline blood sample and medical history, will be taken. **Follow-up:** In total the participant will have 12 contacts with study staff during the trial. All visits listed below: • 1 screening visit with blood draw (any time during a 60 day period before enrolment). Note that there must be 2 contacts during the screening visit to allow a minimum of 72 hours between receiving the oral + written information and signing the consent form. The first of these contacts may be telephonic in which case the information sheet is emailed to the interested party. If the screening visit occurred more than 60 days before the vaccination visit as originally planned, a new medical history of the participant and a complete blood count (2.6 mL) will be carried out in the week preceding the vaccination (D-7 to D-1). Other laboratory tests may be realized if deemed necessary by a study physician according to potential medical events that may have occurred between screening and vaccination. • 2 vaccinations with pre-vaccination blood draws (day 0 and 21) • 3 telephone calls (2 taking place 24 hours after each vaccination: day 1 and 22 and one on day 60). These calls may be transformed into a physical consultation if deemed medically necessary. • 6 physical consultations with blood draws (day 7, 14, 28, 35, 90 and 180) **See Figure 1** for summary schedule, and **See Table 2** for full schedule Blood samples will undergo various safety and immunological testing.

[4] Such as full blood count [FBC], alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin, serum creatinine Urine samples will be tested for the presence of protein, blood, and glucose

[5] Such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV)

	All fevers or flu like illnesses will be assessed for COVID-19. SARS-CoV-2 antibody titres will be taken at several points in the study. Specifically, all fevers ≧38°C, or clinical syndromes meeting local testing criteria in the Canton of Vaud will be investigated for COVID-19 with PCR or antigenic test.
Study Product / Intervention	**Active substance** • PepGNP-Covid19: A synthetic T cell priming setpoint modifying SARS-CoV-2 vaccine composed of ultrasmall carbohydrate-passivated gold nanoparticles carrying covalently bound MHC class I-binding peptides derived from the SARS-CoV-2 ligandome. **Diluent** • Water for injection (WFI, 50 µl)
	Two doses will be tested sequentially in a risk-minimising, dose-escalation strategy: • **Low Dose (LD):** 2.5 nmol total peptide/dose with 12.8ug gold base particle in 50 µl WFI • **High Dose (HD):** 7.5 nmol total peptide/dose with 38.3ug gold base particle in 50 µl WFI
Comparator Intervention	**Substance** • Base Particle Comparator (bpC) comprised of a gold particle **Diluent** • Water for injection (WFI, 50 µl)
	Two dosages will be used to match the GNP dose in LD and HD vaccine vera: • **Low Dose (LD) vehicle-GNP:** 12.8 ug in 50 µl WFI • **High Dose (HD) vehicle-GNP:** 38.3 ug in 50 µl WFI
Administration	Two intradermal injections of the IMP or vehicle-GNP will be administered in the upper arm of each consenting participant. The injections will take place on day 0 (first injection) and day 21 (second/booster injection) using the Nanopass MicronJet600 (https://www.nanopass.com/product/) microneedle.
Number of Participants with Rationale	This study will enrol 26 participants (20 vaccine vera and 6 Base-Particle Comparator [**vehicle-GNP**] split 50:50 between dosage groups as depicted below.  Since this is a first-in-human study with a focus on safety, participant numbers are limited to power the detection of adverse events with high incidence rates. See statistical considerations below.

<table>
<tr><td>Statistical Considerations</td><td>
Having thirteen participants per group (with in total 20 exposed to the investigational product at either dose), would allow 80% power of detecting an AE with a true incidence of:
5% across all exposed participants (LD and HD combined) or
20% within a single dose group (LD or HD)
Achievable statistical power (1-β) to observe at least 1 AE at various incidences (λ) within the investigational sample size
<table>
<tr><th rowspan="2">True incidence of the AE (λ)</th><th colspan="3">Sample size required to detect a single AE at the statistical power (1-β) listed below</th></tr>
<tr><th>50%</th><th>80%</th><th>95%</th></tr>
<tr><td>2.5%</td><td>28</td><td>64.4</td><td>120</td></tr>
<tr><td>5%</td><td>14</td><td>32.2</td><td>60</td></tr>
<tr><td>10%</td><td>7</td><td>16.1</td><td>30</td></tr>
<tr><td>20%</td><td>3.5</td><td>8.05</td><td>15</td></tr>
<tr><td>30%</td><td>1.75</td><td>4.03</td><td>7.5</td></tr>
</table>
KEY
<table>
<tr><td>At least a single dose-specific AE would be detectable at the given probability within a single dose group of 10 participants (either LD or HD)</td></tr>
<tr><td>At least a single exposure-dependent (any dose) AE would be detectable at the given probability across all dose groups of 20 participants</td></tr>
<tr><td>AE not detectable at this probability within the investigational sample size</td></tr>
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<tr><td></td><td>
An <u>interim analysis</u> by the DSMC will be undertaken with at least 7 days of follow-up information on at least 10/13 of the participants in the LD group, according to Figure 2.
The DMSC will review the merits for
Continuing with a second vaccination within the dosage group
Escalating the dose of naNO-COVID from LD→ HD (i.e. enrolling 3 pioneer HD participants)
</td></tr>
<tr><td>Study Duration:</td><td>6 months of enrolment for each participant (180 days)</td></tr>
<tr><td>Study Schedule:</td><td>January 2022: first participant in for naNO-COVID
September 2022: last participant out for naNO-COVID (planned)</td></tr>
<tr><td>Investigator(s):</td><td><u>Overall Coordination</u>
- Principal investigator: Prof. Blaise Genton, MD PhD
- Co-investigator: Dr Mary-Anne Hartley, MD PhD MPH
- Immunological evaluator: Prof. François Spertini, MD
- Project coordinators: Dr Juliette Besson and Dr Alix Miauton, medical physicians</td></tr>
<tr><td>Study Centre(s):</td><td>This is a clinical trial conducted at a single site in Switzerland
- Center for primary care and public health, Unisanté & Clinical Trial Unit, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland</td></tr>
<tr><td>Compensation:</td><td>Financial compensation will be provided for participation (800CHF/participant) and travel costs will also be covered from their place of domicile.
See Table 2 for payment schedule.</td></tr>
<tr><td>GCP Statement:</td><td>This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP (as far as applicable) as well as all national legal and regulatory requirements.</td></tr>
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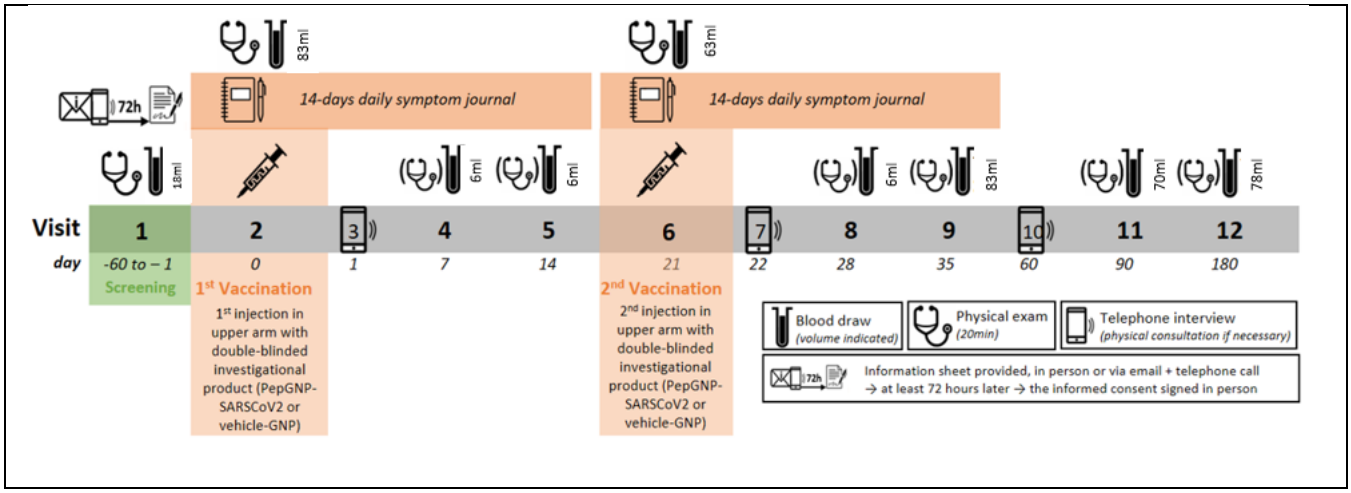

Figure 1: Summarised 180-day schedule of study procedures for a single participant

Table 2: Schedule of study procedures

Visits	1 Screening	2 Vaccination 1	3	4	5	6 Vaccination 2	7	8	9	10	11	12
Timeline (days)	Any time from -60 to -1	0	1	7	14	21	Day 1 after vaccination 2	Day 7 after vaccination 2	35	60	90	180
Tolerance (days)		0	±1	±1	±2	±2	±1	±1	±2	±7	±14	±14
Summary of interventions												
Screening and trial procedures												
1. Inclusion/Exclusion criteria	✓	✓ *(confirmation)*				✓						
2. Informed consent	✓	✓ *(confirmation)*										
3. Medical history	✓	✓ *(confirmation)*				✓				✓	✓	✓
4. Concomitant medication	✓	✓ *(confirmation)*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5. Physical exam	✓	✓ *(confirmation)*	(✓)	(✓)	(✓)	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
6. Compensation (CHF)	0	100	0	100	100	100	0	100	100	0	100	100
Intervention												
7. Study Vaccination		✓				✓						
Safety monitoring *(Safety monitoring is continuous. Opt-in reporting is active on all days throughout study)*												
8. Provide diary card		✓(1st)				✓(2nd)						
9. Collect diary card + photo (if any)					✓(1st)				✓(2nd)			
10. Solicited local reactogenicity		✓	✓	✓		✓	✓	✓				
11. Solicited systemic reactogenicity		✓	✓	✓	✓	✓	✓	✓	✓			
12. Review AE/SAE/AESI		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Samples taken for blood safety tests and serology												
13. Complete blood count, 2,6 mL EDTA	2,6 ml			2.6 ml	2.6 ml			2.6 ml	2.6 ml			
14. Biochemistry, 2.6 mL LiHe	2.6 ml			2.6 ml	2.6 ml			2.6 ml	2.6 ml			
15. HIV, HBV, HCV, 4.9 mL EDTA	4.9 ml											
16. SARS-CoV2 test (PCR/antigenic test)		✓				✓						
17. βHCG, urine test	✓ (urine)	✓ *(pre-vaccine)*				✓ *(pre-vaccine)*						
18. Contraception	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
19. Urinary protein, blood, glucose	✓ (urine)											
20. Anti-nuclear antibodies[a] 7.5 mL serum gel	7.5 ml										7.5 ml	

Samples taken for secondary immunogenicity studies												
21. HLA typing, 4.9 mL EDTA		4.9 ml										
22. PBMC & plasma, 7.5 mL LiHe		67.5 ml *(pre-vaccine)*				52.5 ml *(pre-vaccine)*			67.5 ml		52.5 ml	67.5 ml
23. Antibody tests, 2x4.9 ml serum-gel		9.8 ml *(pre-vaccine)*				9.8 ml *(pre-vaccine)*			9.8 ml		9.8 ml	9.8 ml
Secondary Immunogenicity Outcomes												
24. Anti-SARS-CoV-2 IgG response		✓				✓			✓		✓	✓
25. CD8 T cell response [b]		✓				✓			✓		✓	✓
Exploratory Immunogenicity Outcomes												
26. Ig functional assay [c]		(✓)				(✓)			(✓)		(✓)	(✓)
27. T and B cell responses assessment [d]		(✓)				(✓)			(✓)		(✓)	(✓)
TOTALS												
Summary of interventions												
Daily vol (ml)	17.6	82.2	0.0	5.2	5.2	62.3	0.0	5.2	82.5	0.0	69.8	77.3
Max cumulative vol (ml)	17.6	99.8	99.8	105.0	110.2	172.5	172.5	177.7	260.2	260.2	330	407.3
Compensation cumulative (CHF)	0	100	100	200	300	400	400	500	600	600	700	800

Legend for Table 2

[a] As a surrogate for autoimmune reactions. A 4-fold increase from baseline along with a positive history of clinical signs/symptoms in line with autoimmunity, will be investigated as an adverse event.

[b] Frequency of peptide-specific CD8+ T cells on PBMCs by 2 flow cytometry methodologies: ex vivo, using staining with vaccine specific dextramers, CD3, CD4, CD8 and memory markers (CD45RA, CCR7) at D0, D35 and D180; and using activation-induced markers (AIM) CD69, CD137 and CD107a, in response to peptide stimulation *in vitro*, at D0, D21, D35, D90 and D180.

[c] Serum Ig functional assay, neutralization and enhancement, performed if the vaccine induces anti-SARS-CoV-2 antibodies, as assessed by anti-SARS-CoV-2 serology (24). .

[d] Additional exploratory analyses of Coronavirus-specific cellular responses at various time-points: Additional characterization of the vaccine induced response, cytokine secretion, Coronavirus-specific cell functional assays.

Physical consultation at study site	✓ Performed
Blood draw	(✓) Performed if deemed necessary
	+photo if any In the first 7 days post-vaccination participant will be asked to take a photo of the injection site in the event of signs/symptoms of local reactogenicity
Vaccination	
Telephonic consultation	

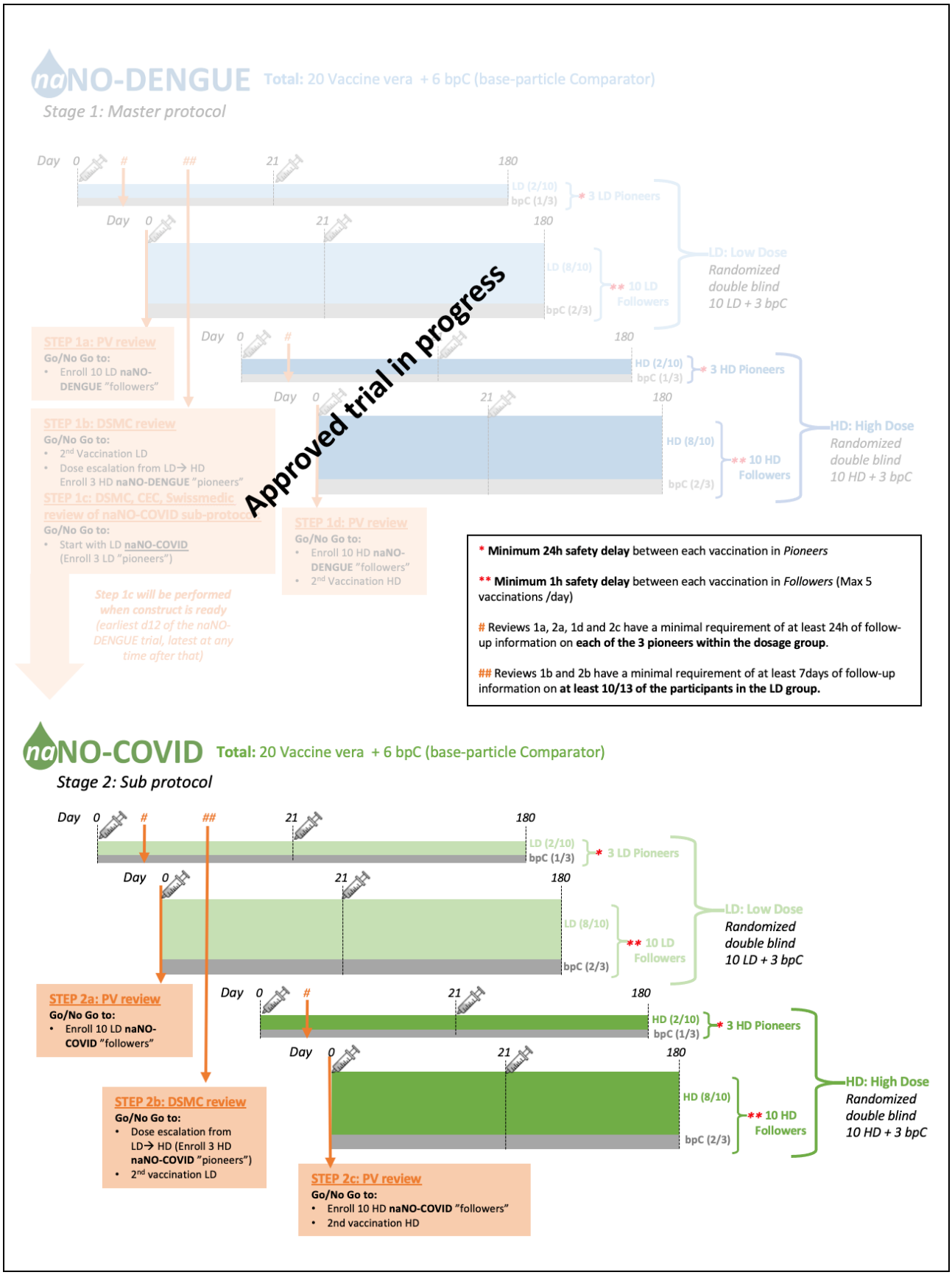

Figure 2. Overview of adaptive trial Stage 1 naNO-DENGUE (Previously approved master protocol. Trial in progress) and naNO-COVID (THIS TRIAL)

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor

1.1.1 Sponsor's main contact person

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1.1.3 Sponsor's Representative in Switzerland

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1.2 Investigators

1.2.1 Principal investigator

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1.2.3 Project coordinators

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1.3 Statistician

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1.4 Laboratory

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Role	Designs and perform immunological assays, Analysis and interpretation Writing of immunological study report

1.5 Monitoring institution

Name	Elisabeth Reus
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Role	Study monitor

1.6 Data Safety Monitoring Committee (DSMC)

Chairperson

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1.7 Any other relevant Committee, Person, Organisation, Institution

1.7.1 Pharmacovigilance

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1.7.3 Regulatory Affairs and Project Management Support

Name:	Clinical Trial Unit (CTU*)* CHUV & University of Lausanne (UNIL)
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In addition, all the physical visits will take place (under the responsibility of the principal investigator) in the clinical investigation unit of the CTU CHUV-UNIL (Bugnon 19, 1011 Lausanne).

2. ETHICAL AND REGULATORY ASPECTS

The decision of the competent ethics committee and Swissmedic concerning the conduct of the study will be made in writing to the Principal investigator/Sponsor's legal representative in Switzerland before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study Registration

This study will be registered in the Clinicaltrials.gov before the study starts.

In addition, the study is registered in the Swiss National Clinical trial Portal (SNCTP via BASEC) in French.

2.2 Categorisation of study

This is a first-in-human phase I safety trial of a new nano-particle vaccine against a virus in a population in which the disease is not endemic. This trial is thus considered of category C according to ClinO.

2.3 Competent Ethics Committee (CEC)

The principal investigator ensures that approval from an appropriately constituted CEC (i.e. Commission cantonale d'éthique de la recherche sur l'être humain du canton de Vaud, CER-VD) is sought for the clinical study.

Any changes in the research activity will be reported by the principal investigator to the lead CEC as per ClinO Art 29. Notifications of immediate safety and protective measures, serious adverse events and annual safety reports are described under **Section 10**.

Premature study end or interruption of the study will be reported within 15 days by the principal investigator to the CEC. The regular end of the study will be reported to the CEC within 90 days and the final study report will be submitted within one year after the end of the study.

2.4 Competent Authorities (CA)

The principal investigator (also acting as the Sponsor's legal representative in Switzerland) will obtain approval from the competent authority (i.e., Swissmedic) before the start of the clinical trial.

Any changes in the research activity will be reported by the principal investigator as per ClinO Art 34. Notifications of immediate safety and protective measures, serious adverse events and annual safety reports are described in **Section 10.**

Premature study end or interruption of the study will be reported within 15 days to Swissmedic. The regular end of the study will be reported within 90 days and the final study report will be submitted within one year after the end of the study by the principal investigator.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance with the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

The investigators in this trial have no conflict of interest to declare.

2.7 Patient Information and Informed Consent

Screening (any day from -60 to -1)

The formal consent of a participant, using the CEC-approved study-specific consent form, must be obtained before the participant is submitted to any study procedure.

The CEC-approved study-specific participant information sheets (PIS) will be made available to the volunteer (either by direct contact or via email with a telephone call for oral explanations) at least 72 hours prior to their screening visit. During this physical or telephonic contact, the volunteer will be fully informed orally of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasized:

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or consequence.
- 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
- The volunteer's general practitioner may be contacted to corroborate their medical history or seek additional information
- The volunteer's blood samples taken as part of the study will be stored in liquid nitrogen and samples may be sent outside of Switzerland to collaborating laboratories. These samples will be identified only by code numbers.
- The aims of the study and tests to be carried out will be explained.
- Financial compensation will be provided for participation (800CHF/participant) disbursed in 8 instalments of 100CHF at each physical visit. Additionally, travel costs will be covered from their place of domicile.
- All relevant clinical and laboratory results (serology, lab tests and interpretations of physical exams) will be shared directly with the concerned participants.

The volunteer will be given a minimum of 72h to make an informed decision about his/her participation in the study. The volunteer should read and consider the statement before signing and dating the informed consent form. The consent form must be signed and dated by the investigator (or their designee) at the same time as the volunteer signs. A copy of the fully signed form is given to the volunteer and the original will be retained as part of the study records.

Enrolment (Day 0)

- Before receiving the vaccine, participants will be reminded of the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail.
- Participants will be reminded that the study is entirely voluntary and that they may withdraw from the study at any time.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual participant medical information obtained as a result of this study is considered confidential and non-anonymised disclosure to third parties is prohibited. Participant confidentiality will be further ensured by utilising participant identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Principal investigator, a competent authority (e.g. Swissmedic), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Principal investigator may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of harm of the experimental intervention

2.10 Protocol amendments

2.10.1 Unforeseen amendments and deviations

2.10.1.1 Substantial and non-substantial amendments

Substantial amendments are only implemented after approval of the CEC and Swissmedic respectively.

All non-substantial amendments are communicated to Swissmedic as soon as possible if applicable and to the CEC within the Annual Safety Report (ASR).

2.10.1.2 Protocol deviations

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human participants may proceed without prior approval of the Sponsor and the CEC/Swissmedic. Such deviations shall be documented and reported to the Sponsor.

2.10.1.3 Safety and protection measures

According to ClinO art. 37, safety and protection measures which must be taken immediately will be notified to CEC and Swissmedic within 7 days.

2.10.2 Prospective amendments

This is an adaptive trial featuring a prospective amendment in the form of this submitted subprotocol (based on the master protocol of NaNO-DENGUE: a previously approved clinical trial under the number: 2020-02258 (naNO-DENGUE_ClinicalTrialProtocol_V4.0_English_20210408_Clean).

3. BACKGROUND AND RATIONALE

3.1 Background

Primary research question. What is the safety and reactogenicity profile of 2 doses of PepGNP-Covid19 nanoparticle vaccine in a small cohort of healthy human volunteers?
Secondary research questions. What are the immunogenicity and *ex vivo* efficacy profiles of 2 doses of PepGNP-Covid19 nanoparticle vaccine in a small cohort of healthy human volunteers?

The SARS-CoV-2 pandemic is ongoing and is far from over. Vaccines are proving effective and flattening the curve in countries across the world. This is a fragile dawn, however, with transmission and deaths still high, unequal access to vaccines (2) and variants of concern (VOCs) threatening to undo progress to date. New VOCs are appearing in different parts of the world and the currently licensed vaccines are not enough to control the virus's continuous spread (3,4). Local and general quarantines and lockdowns are being reinstated, and traveling is still restricted. Data from the AstraZeneca vaccine trial in South Africa highlight the potential for variants such as B.1.351 and P.1 to reduce the efficacy of vaccines (5). Other vaccine data, including those from Novavax and Johnson & Johnson (J&J), show a more modest reduction in efficacy, especially against severe disease caused by these variants (6,7).There is also early evidence of mutations arising independently in the United States that may reduce the efficacy of the licensed vaccines (8).

Several further issues have since emerged with the currently licensed vaccines, especially related to large scale manufacturing production (9), their availability (10), and their safety (the Oxford–AstraZeneca and J&J vaccines remains suspended in a few countries following concerns about blood clots (11,12)). The chairman of the WHO's Strategic and Technical Advisory Group for Infectious Hazards, noted in December that endemicity may be the "destiny" of this virus (13).

Simultaneously, the important role of T cells in the control and protection against coronaviruses in general and SARS-CoV-2 specifically is outlined progressively with new data presented as we move forward (14–16).

Companies and countries worldwide have started to mix and match different vaccines in clinical trials (17) to evaluate if a better prevention profile against SARS-CoV-2 and the emerging VOCs, and even more if a better T cell immunity (18) can be achieved.

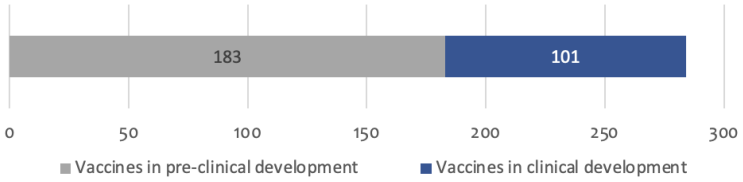
Overall, T cells exhibited distinct differentiation into stem cell and transitional memory states (subsets), which may be key to developing durable protection. Recent reports have also supported an important role for T cell immunity in protecting against COVID-19 in the absence of antibodies to SARS-CoV-2 (19,20).

Emergex's coronavirus vaccine rationale has a unique underlying scientific strategy: the only vaccine that is exclusively inducing cellular immunity / inducing specific T cells against the viruses – and generally a novel kind of vaccine using carefully selected MHC class 1 primer peptides which are experimentally validated, and which are absolutely conserved in all known variants or VOCs of SARS-CoV-2. This vaccine has the potential to complement the other vaccines and fill the gaps in the immunization and prevention strategies.

3.1.1 Rationale for a (-nother) COVID vaccine

Sixty- three days after the SARS- CoV- 2 sequence was published, the first dose of the first experimental vaccine was tested in humans. Fifteen months later, 183 vaccines are in development in 101 clinical trials (21,22). At the time of writing, several COVID vaccines have been approved by the regulatory authorities across the globe and at least three have reported approximately 95% efficacy to infection and moderate disease and complete protection against severe disease. So why does the world need to test a new COVID vaccine? And more specifically, why this one?

The global vaccine alliance, GAVI, has specifically advocated for vaccine diversity as a means to ensure equitable efficacy and access as well as a means of maximizing scalability (23). Several further rationales are presented below.



As of the 23rd May 2021, the WHO reports 183 COVID vaccines in pre-clinical development and 101 undergoing clinical testing. Eighteen of these are in phase 3. (22)

3.1.1.1 *Immunological heterogeneity to protect against mutant escape*

Until recently, SARS-CoV-2 has spread across the globe with little evolutionary selective pressure. The high R0 value, asymptomatic spread and infectious latent period ensured contagion in a universally susceptible population despite a later immune response in the infected individual. Thus, the mutation rate has been relatively slow (24). However, as global immunity rises, so too will the frequency of variants (VOCs) with the potential to escape the prevailing immune response. The risk for escape is particularly high if the population's immunity is homogenous. Thus, there is a need for immunological diversity to protect against vaccine resistance.

3.1.1.2 *Tailoring vaccines to individual needs*

Vaccines are unlikely to be universally efficacious in all populations and demographics. Immune profiles and reactivities change dramatically with age and vary across individuals from anergic to allergic (3). Comparative analysis of several effective vaccines will allow us to tailor vaccine choice to the recipient's immunological and allergic risk profile.

3.1.1.3 *Scaling manufacture*

By diversifying manufacturing methods, there is less risk of global reagent stockouts and large-scale contaminations. Parallelizing production also ensures greater availability sooner.

3.1.1.4 *Democratizing access*

Currently all vaccine candidates require cold chain. In particular, Pfizer-BioNTech requires -70°C (25) which is difficult for low-resource settings (26). While the IMP proposed in this study also requires cold chain, it is structurally plausible for a dry patch preparation in the future.

3.1.1.5 *Diluting monopoly*

As previously feared, high resource settings have been seeding their vaccine stock with high volume pre-order contracts that exceed their need (26). While we hope global distribution agreements such as COVAX (27) will ensure distribution equity, the risk for commercial interest superseding public good is present in monopoly.

The monopoly will have effects far beyond the pandemic and COVID, with enormous financial gains centralised in a handful of companies which could drive future public health research priorities.

3.1.2 Rationale for *this* COVID vaccine

The proposed IMP is a gold nanoparticle coated with T cell-priming peptides against conserved regions of the SARS viruses that are not prone to mutations. This is a unique approach compared to other candidates and offers several advantages for this pandemic and for benchmarking a rapidly scalable platform for emerging viruses.

3.1.2.1 *The need for targeted cellular immunity*

***Humoral immunity may be transient and insufficient*.** While early vaccine efforts focused on antibody responses, evidence is now mounting for the importance of cellular immunity in acute infection and in the maintenance of immunological memory. More importantly, evidence indicates that a robust antibody (Ab) response alone may be insufficient to avoid severe disease and might even promote it (28,29) under certain poorly understood circumstances, raising the possibility of vaccine induced immunopathology (30). For instance, previous studies in animal models have shown that in SARS-CoV infection, anti-S protein-neutralizing antibodies (anti-S-IgG) can also cause severe lung injury by altering inflammatory responses in macaques (31). Further, speculation is that the SARS- CoV- 2 virus–antibody complex could potentially trigger such FcR- mediated inflammatory responses, causing acute lung injury (32). Complicating the picture, is the potentially divergent roles in antibody subtypes. For instance, IgA has been linked to severe COVID-19 (in which the authors attribute to IgA deposition and vasculitis) (33) or have conversely related to recovery (34).

Something more certain is the link between antibodies and the rare clotting disorder triggered by the AstraZeneca vaccine formulation (35) which caused a series of suspensions that had lasting damage to vaccine hesitancy (36). Moreover, antibody based immunity to SARS-CoV-2 may not be so long-lived: Similarly to what was observed for SARS-CoV-1 and MERS-CoV, the antibody levels for SARS-CoV-2 decline in the 6 months following infection (37,38), raising question on their utility as long-lived immunization strategy (39). More specifically, several studies have shown variable and often low antibody titres in convalescent patients, and in particular the very low titres or entire absence of antibody or antibody against SARS-CoV-2 in up to 33% of recovered patients (40–43) and, together, point to a critical role for other immune mechanisms in recovery from the disease (30,44,45).

Cellular immunity is lasting and associated with recovery. In contrast, T cell memory in respiratory coronaviruses was recorded to be long-lived (>6–17 years) (30,46,47) and targeted against epitopes that were less prone to mutation and escape (1). Indeed, studies have shown there to be a particularly robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19 (45). In SARS, cytotoxic CD8 T cells were required for virus clearance and in COVID-19, several clinical studies have identified reduced CD8 T cells as an early prognostic indicator of severe or lethal disease and treatment efficacy, prompting calls that "an effective COVID-19 vaccine needs to engage T Cells" (48). Further evidence of the importance of cellular immunity in COVID is that it is dependent on the HLA haplotype of a person and its capacity to present SARS-CoV-2 epitopes to T cells. In Italy, prevalence of the potentially permissive alleles HLA-B*44 and C*01 correlated with COVID-19 spread (49,50), and thus carefully curated peptide cocktails that fill these immune gaps (such as naNO-COVID) may prove useful to epidemic control.

Finally, intriguing evidence has also detected existing cross-reactive cellular immunity to SARS-CoV-2 in 40–81% of unexposed individuals. This immunity was probably elicited by non-COVID endemic coronaviruses causing the common cold and may contribute to the relative protection of most people against COVID (47,51–54). This is particularly promising for cellular immunity in the face of other emerging coronaviruses. Unfortunately, current vaccine candidates have limited T-cell engagement and were not designed to target the TCR and its various HLA haplotypes. Among all current vaccine candidates with published immune profiles, T cell responses are variable. While the Pfizer-BioNTech mRNA vaccine elicited CD8 T cell responses and T_H1-skewed CD4 T cell responses in > 80% of participants it varied among individuals (55). The Moderna mRNA vaccine elicited T_H1-skewed S-protein specific CD4 T cell but low CD8 T cell responses in most subjects (56). For all candidates, it remains unclear whether the T cell responses are high enough for robust and lasting protection, especially as they only target the Spike Protein, and all fail to leverage the majority of SARS-CoV-2 T cell epitopes that are clearly targeted in naturally infected individuals (48).

This vaccine selects peptides from the SARS viruses ligandome that specifically and exclusively engage the MHC receptor across a range of HLA haplotypes in an effort to elicit a lasting cellular immune response. It also specifically seeks to avoid generating an antibody mediated enhancement of the disease.

3.1.2.2 The advantages of nanoparticle delivery systems

Dose-sparing. Nanoparticle (NP) antigen delivery systems are designed to protect antigens from premature proteolytic degradation and control their release as well as facilitating antigen uptake and processing by antigen presenting cells (APCs) (57). By increasing the effective activity-per-unit, it allows the reduction of effective antigen dose (to nanomoles). Such dose-sparing strategies become critical in scaling production, which is why NP platforms are considered to be highly promising for COVID-19.

Immunomodulation. A more classic dose-sparing strategy in vaccine production, is the addition of an adjuvant. Interestingly, NPs have shown intrinsic immunomodulatory functions, acting as adjuvants or immune potentiators (58). When added for this purpose, they are called vaccine adjuvant nanoparticles (VANs) and are considered to improve the overall efficacy and safety of the generated immune response (59).

Targeted, controlled release. NPs not only improve the immunogenicity and stability of antigen, but also achieve targeted delivery and sustained release (60). Thus, avoiding the high-dose "antigen dump" of traditional vaccines as well as co-localising adjuvant and antigen, to avoid non-specific stimulation. Specifically, gold nanoparticles (GNP) as used in this study have previously been shown to increase inflammatory cytokines both *in vitro* and *in vivo* (59).

Rapidly scalable to new epitopes. Many view the greatest advantage of the NP platform is that it may serve as a "plug-and-play" technology that can be tailored to seasonal or new strains of coronaviruses (61). Indeed, COVID-19 harbours the potential to become a seasonal disease; underscoring the need for continued investment in coronavirus vaccines (62).

3.1.2.3 Gold nanoparticles (GNP)

There are multiple types of NPs, but GNPs hold particular promise in vaccines for cellular immunity. For example, in 2011, Staroverov *et al.* evaluated the protective immune response in mice and rabbits stimulated by a vaccination for a coronavirus known as swine transmissible gastroenteritis virus (TGEV) that was either conjugated or not with an GNP (63). Compared with free antigen, the GNP-conjugate was found to elicit higher concentrations of IFN-γ and 10-fold the T cell division. A more recent study tested the humoral response of an GNP vaccine formulation in mice comprised of S-protein antigens from a murine-adapted SARS-CoV-2 virus (64). Here, both the free antigen and GNP formulations only offered modest antibody-mediated protection in contrast to a formulation with a TLR-adjuvant. The cellular immune response was not evaluated.

The proposed vaccine of this trial specifically seeks to avoid an antibody mediated response by carefully curating an MHC-I binding antigen cocktail from the SARS-CoV-2 ligandome and loading it onto a robust nanoparticle for controlled release and coupled Th1 immunostimulation.

3.2 Previous human and animal experience

3.2.1 Existing COVID Vaccines

At the time of protocol writing, 101 vaccine candidates were registered in clinical trials according to the WHO (22). The candidates have a broad range of approaches, with a third based on protein subunits, another third relying on nucleic acids, (with an even split between DNA and RNA) and another third in replicating and non-replicating viral vectors. Only 13% are based on inactivated virus. Several candidates have been approved by regulatory authorities after showing safety and efficacy against the first circulating SARS-COV-2 variant in fast-tracked phase III clinical trials.

Moderna's mRNA- 1273 vaccine was the first candidate to begin clinical trials in the US on 17 March 2020 with the National Institute of Health's National Institute of Allergy and Infectious Diseases (NIAID) (NCT04283461). This Phase I study involved 45 patient volunteers, divided into three group cohorts, as a dose escalation: low (25 µg), middle (100 µg) and high (250 µg) in a prime boost. For comparison, the IMP proposed in this, naNO-COVID, phase I trial has a similar number of patients (n=26) with a 2-dose escalation (low and high with IMP levels of 2.5nmol and 7.5nmol respectively. The Moderna vaccine proved immunogenic, with dose-dependent antibody titres and 20% of high dose participants reporting severe adverse events, with severity increasing after the second vaccination (56). Phase II (NCT04405076) and Phase III (NCT04470427) trials selected the moderate 100- µg dose, and it was approved in December 2020 along with another RNA-based vaccine from Pfizer-BioNTech, BNT163b2 (NCT04368728). While both offer 95% efficacy, the Pfizer-BioNTech formulation requires a third of the dose (30ug) but requires a storage temperature of -70°C. The latter has since been approved in the EU (65) and Switzerland (66).

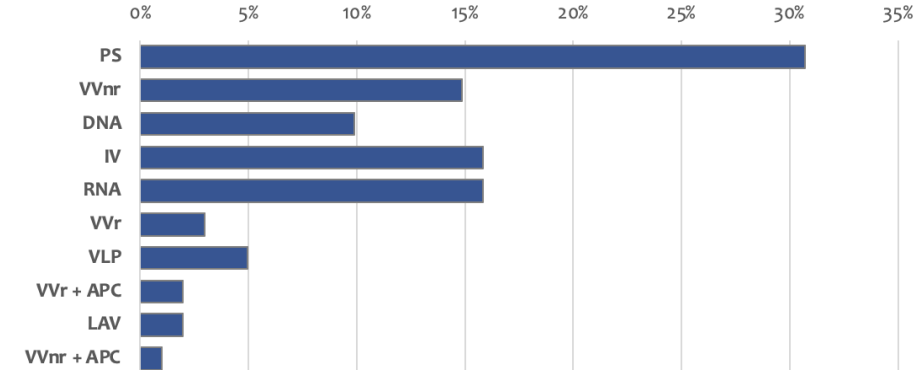
Several other viral vector-based formulations such as the UK-Swedish AstraZeneca (AZD1222) vaccine and the Russian Sputnik V have shown slightly lower efficacy (approximately 90%) against the first circulating SARS-COV-2 variant but their simplified storage conditions (refrigeration only for AZD1222) and price (5-fold cheaper than the Moderna formulation) may be preferred in lower resource settings, which showcases the diversity of vaccines needed in the fight against the coronaviruses.

Regardless of the variety available, it is unlikely that manufacture will scale to meet demand , leaving many questioning how to ensure equitable distribution in the months ahead of short stock (26).

More "traditional" vaccine formulations comprising an inactivated virus have been produced by Sinopharm in the Institutes of Biological Products in Beijing (ChiCTR2000034780) and Wuhan (ChiCTR2000039000), the efficacy against the first circulating SARS-COV-2 variant seems to be inferior to the mRNA formulations and their innate capacity to reactivate (albeit no cases of reactivation were reported) might make it a less favoured option.

Altogether, new VOCs have emerged globally and the need of actual T cell inducing vaccines is more imminent than ever.

COVID-19 vaccines in clinical trials at the end of 2020 according to the platform type.



Platform		Candidate vaccines (no. and %)	
PS	Protein subunit	31	31%
VVnr	Viral Vector (non-replicating)	15	15%
DNA	DNA	10	10%
IV	Inactivated Virus	16	16%
RNA	RNA	16	16%
VVr	Viral Vector (replicating)	3	3%
VLP	Virus Like Particle	5	5%
VVr + APC	VVr + Antigen Presenting Cell	2	2%
LAV	Live Attenuated Virus	2	2%
VVnr + APC	VVnr + Antigen Presenting Cell	1	1%
		101	

COVID-19 vaccines that were either approved or in stage III trials by the end of 2020

Name	Countries	Type of construct	Doses	Efficacy	Storage temperature	Price	Billions of doses by 2021 end (67)
mRNA-1273 (Moderna)	US	mRNA in lipid nanoparticle	2	94.5%	Refrigeration 1 month	$25	1
AZD1222 (Astra-Zeneca)	UK/Sweden	Adenovirus-based (chAdOx1)	2	90%	Refrigeration for 6 months	$4	3
BNT162b2 (Pfizer-BioNTech)	US/Germany	mRNA in lipid nanoparticle	2	95%	Freezer -70°C	$20	1.3
Ad26.COV2-S (Janssen)	UK	Adenovirus-based (Ad26)	1	?	Refrigeration for 3 months	$10	1
Sputnik V (Gamaleya)	Russia	Adenovirus-based	2	91.4%	Freezer -20°C	$10	1
BBIBP-CorV (Sinopharm Beijing)	China	Inactivated	2	86%	?	?	?
Sinopharm Whuan	China	Inactivated	2	?	?	?	?
CoronaVac (Sinovac)	China	Inactivated (beta-propiolactone)	2	91.25% (tentative results)	Refrigeration for ? months	?	?
CanSino Biological	China	Adenovirus-based	1	?	?	?	?
(NVX-CoV2373) Novavax	US	Protein subunit + nanoparticle + saponin adjuvant	2	?	Refrigeration for ? months	?	?

3.2.2 Existing nanoparticle-based vaccines

Nanocarriers can have various base molecules ranging from organic (lipids, proteins) to inorganic (metals or polymers). The major advantages of inorganic NPs include low production cost, reproducibility and favourable safety profiles (68). So far, inorganic gold nanoparticles (GNP) have shown a particularly promising immunological and risk profile in several experimental viral vaccines tested in a murine model (e.g. influenza

(69), HIV (70) and foot and mouth disease). So far, no safety issues have been reported with gold nanoparticles produced by the Midatech technology after oral or intradermal administration in humans or in any animal model. The possible induction of gold allergy after intradermal injection in some T1 diabetes patients wasn't considered prohibitive to continue dosing in those patients by the regulatory authorities in the UK. NP have also already been used in various human vaccine preparations requiring a cytotoxic CD8 T-cell response. In particular, an NP-based melanoma vaccine containing a CpG adjuvant was tested in a phase I/II study hosted at the same site in Lausanne as this proposed trial. The trial showed that the vaccine was well tolerated, and a majority of patients generated appropriate *ex vivo* T-cell responses (71).

3.2.2.1 Nanoparticle vaccines for COVID

NP technology has been used in several of the approved vaccines. Indeed, both the Moderna and Pfizer-BioNTech formulations encapsulate their mRNA sequences in lipid nanoparticles in order to deliver them intracellularly for replication. One “true” nanoparticle vaccine (whereby protein subunits are mounted on a nano-structure) is currently undergoing phase III clinical trials. The NVX- CoV2373 formulation by NovaVax (72) comprises a recombinant nanoparticle that displays the SARS- CoV- 2 spike protein and their own saponin- based Matrix- M adjuvant (NCT04368988), the latter of which has proved essential for 100% seroconversion (72). As with all the other COVID vaccine candidates with published evidence, two doses were required for neutralising antibody in all individuals. Additionally, a modest cellular immune response was seen in several individuals, but the protective relevance of this is unknown.

3.2.3 Existing T Cell-Specific Vaccines

Two of the most effective immunizations known to medicine, smallpox vaccine (*Dryvax*) and yellow fever virus (YFV)-17D vaccine, are based on an MHC class I-specific mechanism. Smallpox is an exemplar for how effective vaccination can result in disease elimination. In the United States, the last reported case occurred in 1978 and (with the exception of exposed laboratory workers and military personnel), smallpox vaccination was discontinued in 1972. While the yellow fever vaccine is similarly effective at preventing infection, reservoirs of sylvatic transmission and insect vectors have thwarted elimination efforts. The vaccine is currently limited to inhabitants and visitors of high-risk areas. Both *Dryvax* and YFV-17D are based on live virus formulations that result in acute infections with viral replication and a subsequent long-term protective immunity based on an MHC class I immune response (73–75). This immunological memory is established by a pool of memory $CD8^+$ T cells (76) that express the IL-7 receptor (CD127) (77) and that persists for several decades (78). These antiviral $CD8^+$ T cell responses were shown to peak around two weeks post immunization with around 12.5% and 40% of peripheral $CD8^+$ T cells displaying an activated phenotype ($CD38^+$, HLA-DR^+, Ki-67^+, Bcl-2^{low}) in YFV-17D and *Dryvax*, respectively, with no detectable evidence of bystander activation (79). Phenotypic analysis of vaccinia virus-specific $CD8^+$ T cells revealed that all effector T cells expressed perforin and granzyme B at the peak of the response. The contraction and memory phase of the response was associated with further differentiation of vaccinia virus-specific T cells. Together, the data support a model of human $CD8^+$ T cell differentiation in which naive $CD8^+$ T cells undergo massive expansion in response to antigen and pass through an effector phase prior to gradually differentiating into long-lived memory cells.

3.3 Investigational product (COVID Vaccine Candidate) and indication

3.3.1 The vaccine candidate: PepGNP-Covid19

In light of the risks posed by the development of disease-enhancing antibodies and live, genetically modified COVID compositions, Emergex has developed a potentially antibody-independent vaccine candidate. PepGNP-Covid19 is composed of synthetic T cell-selective multivalent COVID (SARS-CoV-2) peptides carried on carbohydrate-passivated gold nanoparticles (GNP). The COVID peptides were selected from an expression library of experimentally determined HLA supertype[6] epitopes[7] that have specificity to MHC class I[8] receptors on T cells. This aims to eliminate the risk of infection from vaccine products and minimise the risk of vaccine-induced disease enhancement.

Below we describe these two components 1) the peptides and their selection and 2) the gold nanoparticle delivery system

[6] **HLA supertype:** HLA groups with largely overlapping peptide binding specificities
[7] **HLA/MHC epitope:** The part of the peptide molecule to which the HLA molecule binds
[8] **MHC class I:** molecules with the function of presenting peptides to cytotoxic T cells

3.3.2 Peptide Selection

The peptide selection has been manufactured to GMP quality standards and validated in both pre-clinical and GLP toxicology models; and is thus in line with Phase I development standards.

The "ligandome": To produce a cell-mediated COVID vaccine, it is necessary to first identify the MHC-I binding peptides (ligands) that are expressed within conserved regions of the SARS- viral family. These peptides were identified using an immunoproteomics approach that has already been validated for a range of viral indications including Dengue, Influenza and Hepatitis. Briefly, an HLA-typed human cell line is infected *in vitro* with the relevant virus, after which the peptides expressed on the surface of the infected cells are extracted and identified as the MHC-I "ligandome." The resulting library of peptides represents the repertoire of viral ligands that the immune system selects for the generation of an MHC-I mediated response against the virus. The library was then investigated for eligible vaccine candidate epitopes which would be able to prime a cross-reactive immune response against SARS-CoV-2 infection.

Peptide selection strategy: The eight peptides selected from the SARS viral family ligandome, are prepared as a quasi equimolar mix (i.e. 1:1:1:1:1:1:1:1) and detailed in the IMPD. These represent conserved internal peptides that are not prone to mutation. The concentration indicated for each dose group refers to the total concentration of all 8 peptides taken together with their GNP carrier molecules.

The peptides are selected according to the following rationale and criteria:

a) Cross-reactivity between strains based on sequence similarity: Peptides from the internal nucleocapsid protein were favoured, as they are generally better conserved within the viral family. One of the 8 peptides is from the spike protein.
b) Cover particular HLA supertypes: a greater selection of HLA types confers greater population coverage. For this vaccine, 7 HLA, HLA-A2, -A24, -A3, -A29, -B7, -B35 and -B44, are included which is predicted to provide >95% coverage.
c) Multiple protein/peptide coverage: An optimal T-cell vaccine would promote multi-target pathogen recognition (with epitopes from various protein structures on the virion). Therefore, multiple peptides for each HLA type were selected.
d) Ease of manufacture: In general, the more hydrophobic the peptide, the more complex the synthesis and conjugation with the nanoparticle carrier system. Therefore, more hydrophilic peptides were given preference when possible.
e) Physio-chemical properties: from an efficacy, cost utility and quality control perspective, it is important that the peptides have the following properties: when bound to the carrier-system, they should not aggregate with themselves (i.e. size <6nm) or other peptides in the vaccine, they should also maintain good solubility and have an acceptable peptide : GNP ratio.

3.3.3 Gold nanoparticle delivery system:

Alone, the above-mentioned peptide epitopes would be immunologically weak when administered *in vivo* and would not produce a T-cell response sufficient for an effective clinical vaccine. Free peptides within the body are subjected to proteolytic degradation and are not efficiently delivered to antigen presenting cells (APCs). GNP technology can overcome these issues. By attaching the viral peptides along with specific carbohydrates to a GNP core (circa 1.7 nm), a vaccine construct can be produced that is immunogenic, protective against proteolytic degradation, and able to efficiently deliver the viral peptides to APCs. This will produce a vaccine that, when administered directly to the dermis via an appropriate device, is capable of delivering the immunologically relevant peptides to the specifically targeted location (*i.e.* dermal APCs), in turn inducing a strong T-cell response. Proof of concept utilizing this system has been demonstrated for pathogens with existing ligandome information (please refer to the relevant section in the Investigators Brochure), thereby providing confidence for its application to COVID vaccine production. This is the first time that this platform is being applied for use in COVID.

3.3.4 Administration

Due to the low dosage required, the vaccine candidates are suited to be delivered by intradermal delivery devices. The use of a microneedle is indicated to reduce the incidence of trauma-associated adverse local events and discomfort to the volunteer. In the future this could take the form of novel microneedle skin patch technologies, reducing the training requirements of medical professionals and perhaps allowing vaccinations to take place outside central health clinics.

The development of a dry-patch formulation is intended in the next phase of development.

Vaccinations will be administered to eligible volunteers by microneedles intradermally in the deltoid area of the upper arm. The microneedle used is described in **Figure 3** below adapted from Qiu et al (80).

The needle used in this trial is the Nanopass MicronJet600 (https://www.nanopass.com/product/) which has already been used in a Phase 1 trial for a similar GNP-peptide product for diabetes (https://clinicaltrials.gov/ct2/show/NCT02837094?term=02837094&rank=1).

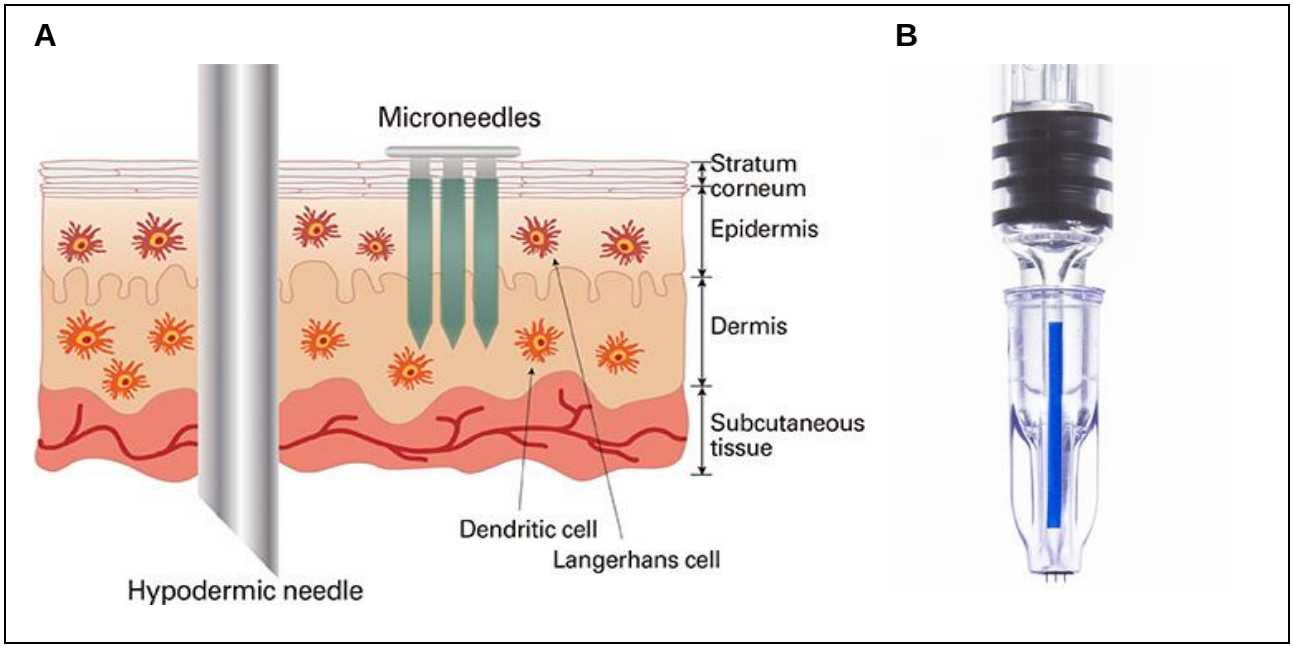


Figure 3. *Microneedle dermal penetrance in comparison to a hypodermic needle (A) and the MicronJet600 needle by Nanopass used in this trial (B)*

3.4 Experimental experience of vaccine candidate

Safety and immunogenicity in vitro and in vivo

For the present clinical trial application for a First-In-Human (FIH) study to assess the safety of peptide-GNP T-vaccines against SARS-CoV-2 virus in human volunteers a number of non-clinical toxicology studies have been performed according to the WHO Guidelines for nonclinical evaluation of vaccines (2005).

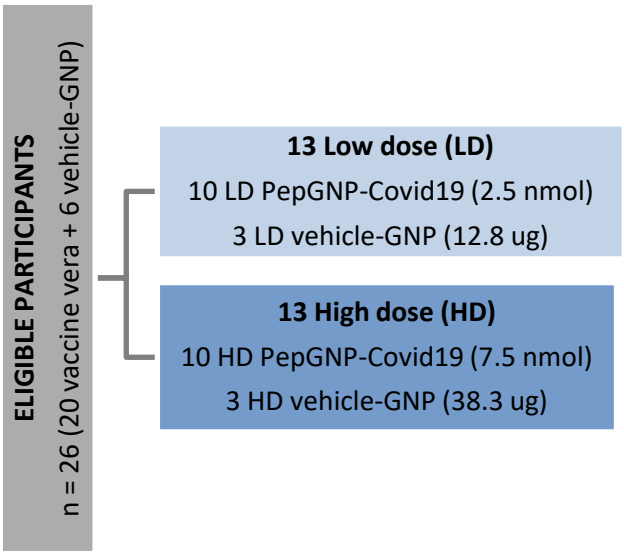
The base carrier has been shown to be safe at the proposed clinical doses in repeat dose toxicology studies following subcutaneous delivery in rats (Study 886.341.5682 reference attached to IB) and rabbits (Study 886.342.5683 reference attached to IB).

The candidate vaccine has shown to be safe at the proposed clinical doses and using the same regimen for administration (2 doses delivered via microneedles i.d.) in GLP toxicology studies in animal models (Study 2810727 in mice and Study 886.342.5941 in rabbits, references attached to the Investigator's Brochure). Preclinical efficacy studies include in vitro (utilising human PBMCs) and in vivo (mouse) studies. Finally, high doses of the COVID vaccine to be used in this trial were tested in a minipig model (a notoriously sensitive animal model in which to study allergic and pseudo-allergic reactions). No evidence of local irritability or hypersensitivity reactions were reported. Further details of these studies are reported in the IB.

3.5 Dose Rationale

This trial evaluates 2 dosages low and high (LD and HD respectively) implemented in a dose escalation strategy after interim safety analyses

These doses were selected based on meticulous preclinical testing of the vaccine constructs. Research has also shown that peptide vaccine trials' doses so far with 0.1 mg, 0.5 mg, up to 3 mg per peptide are all well-tolerated.



This trial will host a LD of 2.5nmol of PepGNP-Covid19 conjugated to 12.8ug of GNP and a HD of 7.5nmol of PepGNP-Covid19 conjugated to 38.3ug of GNP.

Overall, it appears that peptide loaded DCs prime the full spectrum of antigen-specific T cells, while selection of low and high avidity responses is a property of non-professional antigen presenting cells or recall (boost) responses (81).

Therefore, two doses of the vaccine will be given (1+1) in this trial.

3.6 Explanation for choice of comparator

This trial uses a base particle comparator (bpC) arm, as a "vehicle control" with the nano-shell (gold particles without peptide), randomised 10 IMP: 3 bpC. A vehicle control was selected in order to allow a better assessment of the attributes of the base particle itself, and better assessment of the specificity of the peptides when added to the base particle.

The bpC will be provided in 2 concentrations that match the GNP dose in the IMP. The LD bpC will be 12.8ug of GNP and the HD will be 38.3ug of GNP.

3.7 Risk-Benefit Analysis

3.7.1 Potential risks and mitigation strategies

a) Vaccine-induced COVID-19

This is a synthetic peptide and has no potential to reactivate into a disease-causing pathogen.

b) Allergic reactions

Allergic reactions are possible with exposure to any antigen. This includes the antigens of SARS-CoV-2 used in the vaccine preparation as well as to the heavy metal (gold) nanoparticles. Given practical and theoretical evidence, this risk is not estimated to exceed that of currently used vaccines. Indeed, as the vaccine is engineered to present antigen to MHC class I receptors and to *not* produce antibodies, it is theoretically less likely that they will act as a hapten, produce allergy-mediating IgE or engage directly with mast cells. There are also no adjuvants or excipients, which are often the source of vaccine allergies.

As the severity of many allergies is dose-dependent, this trial uses a risk-adverse dose-escalation strategy to minimise the risk of severe allergy and to identify allergic potential. The risk is further mitigated by the WI of the trial unit, providing anaphylaxis-specific continuous monitoring for 60-minutes after each vaccine administration.

c) Local adverse events

Most local adverse events of vaccines (at the vaccination site) are caused by traumatic intramuscular needle injury. This trial makes use of microneedles which limit trauma to the dermis and distribute vaccine delivery over a wider area to minimize tissue damage.

A few naNO-DENGUE participants experienced redness and induration at the injection site, without associated pain or itching, sometimes after sun exposure in the days following vaccination. Due to the possible risk of sun sensitization, it is recommended as a precaution not to expose the vaccinated area for 7 days after vaccination (protection with clothing or SPF 50 sunscreen). Local redness and induration tended to persist for some time, up to months.

Finally, due to the gold nanoparticles, the vaccine is dark brown in color. In some naNO-DENGUE participants,

a very slight grey/brown skin discoloration was visible after vaccination, especially on pale skin. This discoloration disappeared spontaneously, usually within 24 hours.

d) Interaction with SARS-CoV-2 natural infection

By requiring physical visits in a health facility, the patient may be exposed to SARS-CoV-2 infection. However, the participants will attend the CTU in a location that is not part of the out- or in-patients building, which means that the risk of exposure to SARS-CoV-2 **natural infection** will be the same as that in normal life, or even lower.

Further, strict IPC (infection prevention control) measures will be put in place at all meetings as per hospital instructions and as many as possible interactions will be made telephonic (for instance the first of the 2 screening visits may be replaced by emailing the PIS and discussing queries over the telephone).

Nevertheless, running a trial during the COVID-19 pandemic may influence trial results by introducing heterogeneity and febrile disease and immunity from natural infection may be mistaken for that induced by the vaccine.

To address this, regular serologies for anti-SARS-CoV2 antibodies as well as their subtype (IgA, IgM, IgG) will be performed before the vaccination as well as at several points afterwards. Immunological tests will be performed to differentiate Ab specific to the vaccinal peptides from those acquired in natural infection.

Quantitative analyses of existing anti-SARS-CoV-2 Ab will determine whether the vaccine elicits expansion on pre-existing immunological memory.

3.7.2 Potential benefits

The informed consent will clearly state that there are no immunization benefits anticipated on an individual level. Rather, the benefits are anticipated on a population level, for the advancement of a potential COVID vaccine and novel sustainable vaccine development platform. The participants will provide critical information on the performance and safety of the vaccine, which could not only lead the way to advance the trial to its next phase, but also to evaluate the safety and immunogenicity of a vaccine made on a development platform which could be expanded to include other emerging diseases and cancers.

The participant will also receive relevant results of their laboratory tests and may find benefit from this information.

3.8 Justification of choice of study population

This study includes healthy men and healthy, non-pregnant, non-breastfeeding women between the ages of 18 and 45 years old who are residing in Switzerland.

Rationale. As a phase 1 trial, healthy participants are essential to study adverse events. As the effect of the vaccine is unknown on the unborn foetus, we exclude women of childbearing potential[9] by the precautionary principle.

[9] An individual who does **not** have childbearing potential is defined as a female who is:

- Pre-menarche or post-menopausal for at least 1 year
- Surgically sterile
- Using an effective method of contraception from at least 4 weeks prior to the first vaccination until at least 10 weeks after the last vaccination (up to day 90). Effective contraception methods are described in the appropriate section.
- Has no heterosexual intercourses.

4. STUDY OBJECTIVES

4.1 Overall objective

The overall objective is to evaluate the safety, tolerability and reactogenicity of two different doses of the investigational COVID nanovaccine (PepGNP-Covid19) administered to healthy volunteers.

4.2 Primary objective

To evaluate the safety and reactogenicity of two intradermal injections of two different doses of the investigational SARS-CoV-2 peptide T cell inducing vaccine (**PepGNP-Covid19**) administered to healthy volunteers as a

1) candidate vaccine for the prevention of COVID-19

2) proof-of-concept for a rapidly scalable modular peptide vaccine platform

4.3 Secondary objectives

1) To assess the evidence of a CD8 T-cell mediated immune response as a surrogate of protection against severe COVID disease using a novel peptide set point vaccine in healthy adults.

2) To assess the presence of an antibody mediated response

4.4 Exploratory objectives

- To describe any antibody response against SARS-CoV-2 and assess eventual neutralizing or enhancing antibody response if positive for the tests mentioned above.
- To describe the SARS-CoV-2 specific T and B cell responses

4.5 Safety objectives

- Safety is the primary objective of this trial.

5. STUDY OUTCOMES

5.1 Primary outcome measures

The occurrence, nature, time of onset, duration, intensity, seriousness and action taken for any adverse event (AE) reported at any point in the trial (whether it be solicited or unsolicited).

Additionally, the causal relationship to vaccination will also be investigated for any unsolicited event.

Specifically, the:

- Occurrence of solicited local reactogenicity signs and symptoms
  [Time Frame: 7 days following each vaccination]
- Occurrence of solicited systemic reactogenicity signs and symptoms
  [Time Frame: 14 days following each vaccination]
- Occurrence of unsolicited adverse events
  [Time Frame: 6 months following enrolment *i.e. entire trial period*]
- Occurrence of serious adverse events (SAEs)
  [Time Frame: 6 months following enrolment. *i.e. entire trial period*]
- Occurrence of adverse events of special interest (**Section 10.1.1.3**)
  [Time Frame: 6 months following enrolment *i.e. entire trial period*]
- Change from baseline for safety laboratory measures
  [Time Frame: 6 months following enrolment *i.e. entire trial period*]

Note: all AEs will be recorded throughout the study according to Swiss ClinO ordinance. The timelines above refer only to outcome measures.

5.2 Secondary outcome measures

Periodic blood sampling will be undertaken according to the visit schedule in **Figure 1** for the following immunogenicity assessments:

5.2.1 Assess cellular immunogenicity of the candidate vaccine (PepGNP-Covid19):

- Change from baseline of the frequency CD8+ T cell specific to PepGNP-Covid19, proportion of participants with a positive response and characterisation of the memory phenotype of the response.
  [Time Frame: 6 months following enrolment]

5.2.1.1 Specific measures to assess evidence of CD8 T cell response:

- Frequency of CD8+ T cells specific for individual CTL peptides by flow cytometry, using *ex vivo* MHC I dextramer staining for SARS-CoV-2 specific epitopes and memory markers, at D0, D35 and D180;
- Frequency of CD8+ T cells specific for individual CTL peptides by flow cytometry, using activation-induced markers (AIM) CD69, CD137 and CD107a, in response to peptide stimulation *in vitro*, at D0, D21, D35, D90 and D180.

5.2.2 Assess humoral immunogenicity of the candidate vaccine (PepGNP-Covid19):

Assess the humoral immunogenicity of the candidate vaccine (PepGNP-Covid19):

- (For individuals seronegative at enrolment) Anti-SARS-CoV-2 antibody titres and proportion of participants becoming seropositive.
- (For individuals seropositive at enrolment) Anti-SARS-CoV-2 antibody titres and fold change from baseline.

[Time Frame: 6 months following enrolment *i.e. entire trial period*]

5.2.2.1 Specific measures to assess vaccine humoral immunogenicity

- **Anti-SARS-CoV-2 Ig:** Serology to detect antibodies against recombinant (N and S proteins) and/or natural SARS-CoV-2 (lysate or inactivated viral particles)*;* at D0, D21, D35, D90 and D180.

5.3 Other outcomes of interest

5.3.1 Exploratory outcome measures

- Serum Ig functional assay: neutralizing or enhancing antibody response (PRNT or cytometry) for any timepoint at which an antibody response is found
  [Time Frame: 6 months following enrolment]
- Additional characterization of the vaccine induced response, cytokine secretion in response to vaccine peptide stimulation, Coronavirus-specific cell functional assays.

  [Time Frame: 6 months following enrolment]

6. STUDY DESIGN

6.1 General study design and justification of design

After enrolment at the end of a screening visit, participants will be required to attend a total of eleven further follow-up visits/calls over the 180-day follow-up period as detailed in **Table 2** and summarised in **Figure 1**.

The study visits and procedures will be undertaken by one of the clinical trial team members delegated by the principal investigator. All the physical visits will take place at the CTU CHUV-UNIL, Bugnon 19 in Lausanne. The procedures to be included in each visit are documented in the schedule of attendances. Each visit is assigned a time-point and a window period within which the visit will be conducted. Due to reflexion time, the screening visit will take place over several days. Deviations from the visit windows in completing study visits are discouraged but are permitted at the discretion of the investigator (or designee) in the interest of completing the study schedule and obtaining participant safety and immunogenicity evaluations. The tolerance limits to these deviations are listed in **Table 2**.

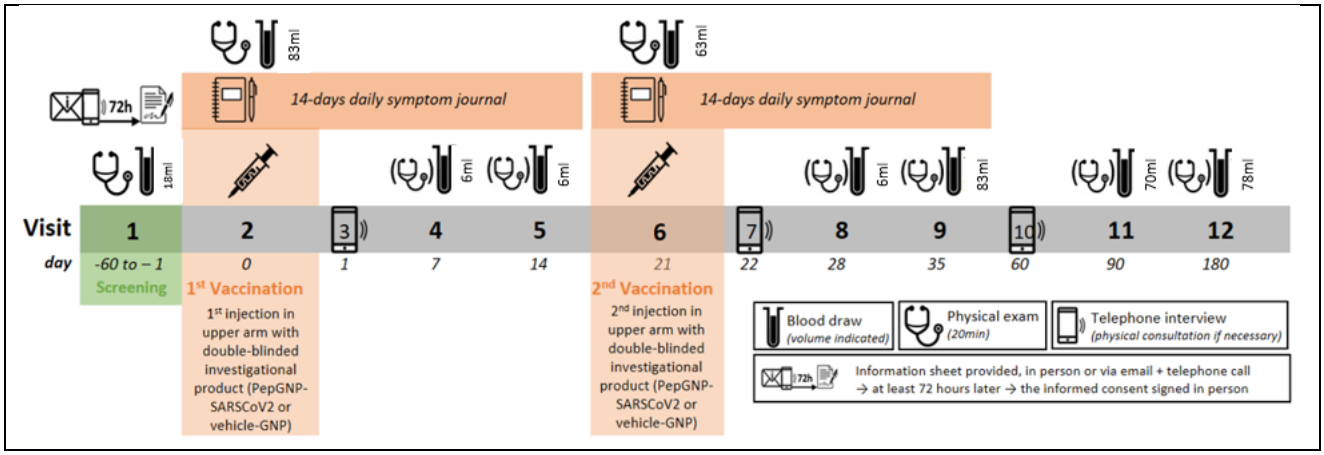


Figure 1: Summarised 180-day schedule of study procedures for a single participant (NaNO-DENGUE and naNO-COVID schedules follow same format)

(copied here for clarity)

6.1.1 Intervention allocation

- **Double-blinded.** Blinded to participants and investigators. Blinding to participants is necessary to ensure that the behaviour (health-seeking, symptom reporting) is not influenced in either arm.
- **Base Particle-controlled.** This trial uses a comparator (Base particle) arm, randomised 3:10. Due to the evolving COVID pandemic, each arm has a temporally matched comparator arm.
- **Safety analysis.** This trial is powered only to detect major adverse events with a prevalence of over 10%
- **Randomised 3:10 (comparator: intervention).** This is a phase I trial, where exposure must be minimised, and this represents the minimum participant level. The nano-shell vehicle is used for comparator to further minimize risk.

6.1.2 Setting

- **Switzerland.** There is no region in the world which is not at risk of COVID, however, conducting this trial in a high-resource setting during a larger COVID-19 vaccination campaign is possibly the most that the risk of getting natural SARS-CoV-2 infection can be minimized.
- **Timing concerns for COVID-19.** Regular SARS-CoV-2 serology will be performed throughout the trial. Currently,around 70% of the population in Switzerland is vaccinated against SARS-CoV-2. The exclusion criteria clearly outline the acceptable delays for non-IMP vaccinations (4 weeks before the first dose of IMP and 4 weeks after the last dose of IMP). Like in most of the clinical trials (and in particular even for approved and licenced first generation vaccines against SARS-CoV-2) during the COVID-19 pandemic, Phase 1 excludes participants with likely current COVID-19. In Phase 2/3, temporary delay criteria defer vaccination of participants with symptoms of potential COVID-19.

6.1.3 Dosages

- **2 dosages with dose-escalation strategy.** To best assess safety, tolerance and (secondarily) efficacy, a dose-dependency is critical to establish possible causality. If there are adverse events, they are expected to be exacerbated at higher doses, thus by first performing an interim safety analysis on the lower dose, we compartmentalise risk and proceed in an evidence-based, externally validated manner.
- **2 vaccinations per participant.** Booster vaccinations are given at day 21 to reduce the risk of drawing false conclusions of safety or poor-immunogenicity which could occur if the vaccine is poorly administered by chance.

6.1.4 Adaptive design

- This is the second stage sub-protocol for a 2-stage study investigating the safety of 2 vaccines from a peptide-based T cell inducing vaccine platform for emerging diseases
  - **Stage 1:** naNO-DENGUE (trial in progress)
    A Phase-I study of a nanoparticle-based peptide vaccine against Dengue virus (Master protocol)
  - **Stage 2:** naNO-COVID **= THIS DOCUMENT**
    A Phase-I study of a nanoparticle-based peptide vaccine against SARS-CoV-2 (Sub-protocol, implemented after interim safety analyses)

6.1.5 Timing

- **6 months follow-up.** The duration of the study for each participant is 6 months. This is an adequate follow-up period for many phase I vaccine trials.

6.2 Methods of minimizing bias

6.2.1 Randomisation

A total of 26 eligible participants will be randomized into the following groups:

- Group 1 (n=13): 10 Low Dose (LD) PepGNP-Covid19 (2.5 nmol) + 3 Comparator
- Group 2 (n=13): 10 High Dose (HD) PepGNP-Covid19 (7.5 nmol) + 3 Comparator

Thus, 20/26 vaccine vera and 6/26 Comparator controls. Allocations of vaccine vera vs Comparator for each group are double-blinded.

The Pioneer group will be randomised by block of 3, while the 10 participants in the follower group will be randomised by block of 5.

Randomization will be performed by the pharmacist.

6.2.2 Blinding procedures

- This trial is double-blinded (blinded to investigators and participants)
- Blinding will be maintained for the duration of the study.
- Bulk concentration of PepGNP-Covid19 and Comparator will be supplied to the CHUV by Emergex, where the vaccines are formulated and vialed. Reconstitution and dose dilution will be performed by a pharmacist at the CHUV and transferred to ready-for-use blinded vaccine vials. These will be labelled with the coded participant ID and certified for release by the pharmacist.
- All allocations will remain coded to all volunteers and investigators. An independent pharmacy team at the CHUV will label the vaccine and Comparator doses with coded participant numbers but will not have access to the identifier list linking the code to the participant identity. All vaccine and Comparator doses will be prepared and labelled away from investigators and stored in identical conditions.
- The appearance of the comparators and doses will be identical. The solutions of both are indistinguishable within the dosage group and thus no shielding of the solution colour is needed.

6.2.3 Other methods of minimizing bias

While the allocation of vaccine vera vs comparator will be double blinded and randomised, the allocation between LD or HD groups and the allocation to pioneer or follower groups will not be double blinded as the schedule reveals the allocation (i.e. the first vaccinated group = LD pioneer). However, the volunteer's position

in the schedule will not be shared with the volunteer and they will thus be single blinded to the allocation of dosage (HD or LD) and to the allocation to the pioneer or follower group.

6.3 Unblinding Procedures (Code break)

- This trial is double-blinded.
- Blinding will be maintained for the duration of the study.

6.3.1 Unblinding during the trial

- The PI or Delegate will unblind any participant if deemed medically necessary for clinical management
- In case of a SUSAR, the PI or Delegate must unblind the concerned participant.
- At the request of the DSMC (on a case-by-case basis for SAE and when a holding rule is activated), the DSMC statistician or Delegate will unblind the participant(s) concerned. The decision has to be transcribed within the committee meeting minutes.

6.3.2 Unblinding at the end of the trial

- Allocations will be revealed once the trial has been completed and the database locked.
- Unblinding will also be performed in case of premature termination of the trial.

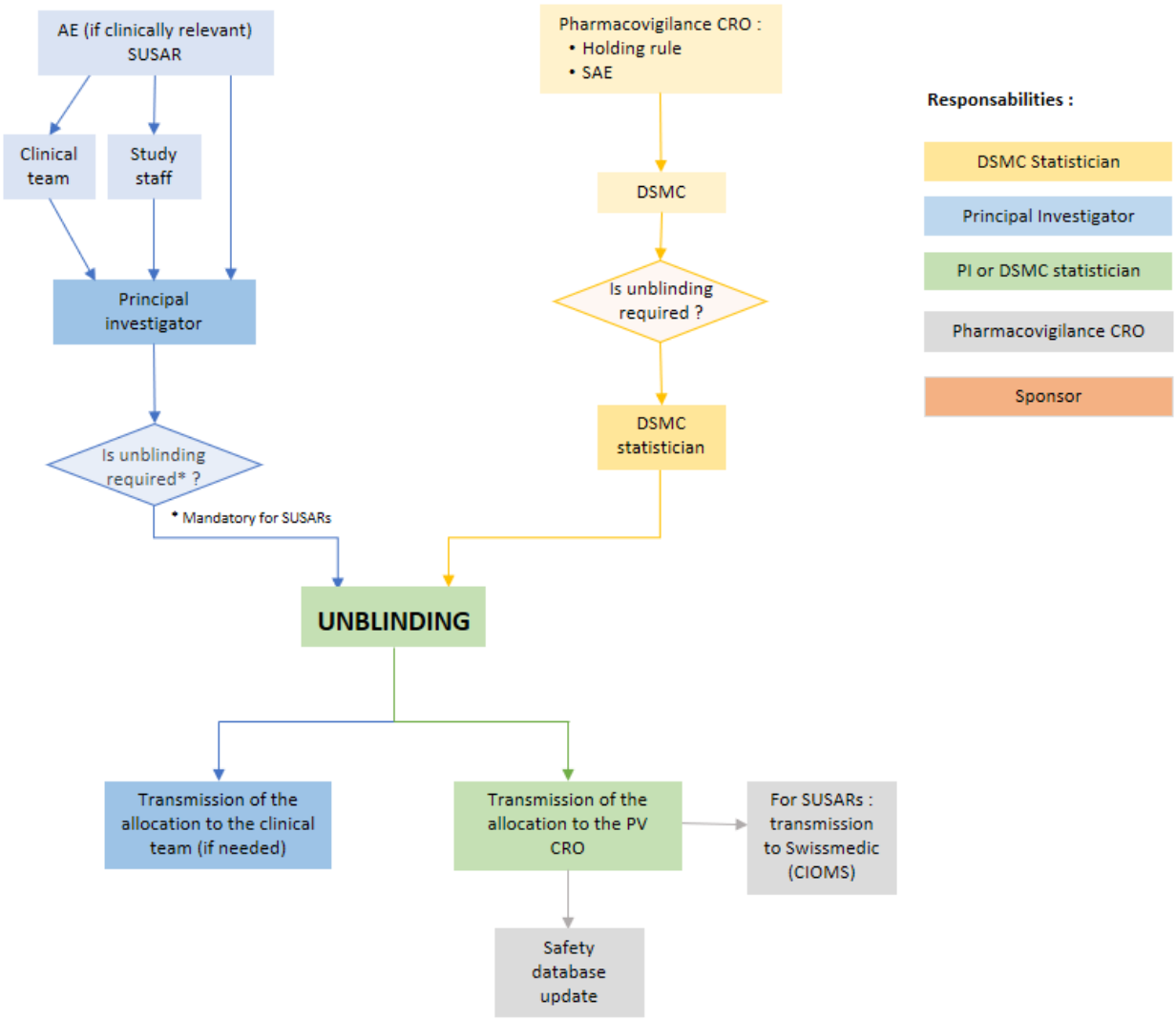


Figure 4: Unblinding reporting

7. STUDY POPULATION

7.1 Eligibility criteria

This study includes healthy men and healthy, non-pregnant, non-breastfeeding women between the ages of 18 and 45 years old who are residing in Switzerland.

Inclusion criteria:	An individual must fulfil <u>all</u> of the following criteria in order to be eligible for trial enrolment: 1. Aged 18 to 45 years on the day of inclusion 2. Participant signed informed consent 3. Residing in Switzerland
Exclusion Criteria	**An individual fulfilling <u>any</u> of the following criteria is to be excluded from enrolment:** 1) Participant is pregnant, lactating, or of childbearing potential[10] 2) Participation in the 4 weeks preceding the first trial vaccination or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure 3) Receipt of any vaccine (including vaccination against COVID) in the 4 weeks preceding the first trial vaccination (excepting influenza vaccination, which may be received up to 2 weeks before first study vaccine) or planned receipt of any vaccine in the 4 weeks following each trial vaccination. 4) Documented COVID-19 disease in the 4 weeks preceding the first trial injection. 5) Receipt of immunoglobulins, blood or blood-derived products in the past 3 months 6) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy[11] 7) Self-reported or documented seropositivity for human immunodeficiency virus (HIV), hepatitis B natural infection (HBcAb positive serology), or hepatitis C 8) Known systemic hypersensitivity to any of the vaccine components (e.g. gold), or history of a life-threatening reaction to vaccines. 9) Current alcohol abuse or drug addiction (reported or suspected) 10) Chronic illness that, in the opinion of the investigator, is at a stage where it might interfere with trial conduct or completion (i.e. any risk factor for a severe COVID-19 disease including BMI > 30 kg/m2) 11) Thrombocytopenia or any coagulation disorder 12) Identified as an Investigator or employee of the Investigator or study centre with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study (i.e. in the employment of the Tropivac clinic or DFRI unit at Unisanté). 13) Refusal to be informed in the event that relevant results concerning the participant's health are revealed
Exclusion Criteria at the Time of Vaccination *(where delayed administration is possible)*	The following events constitute contraindications to the administration of the investigational product on the day of planned vaccination. The participant must be followed until resolution of the event as with any medical event and may be considered for vaccination at a later date (maximum 14 days later) or withdrawn at the discretion of the Investigator. **Delays due to these events do not constitute a protocol deviation.** • Temperature of >37.5°C at the time of vaccination

[10] An individual who does **<u>not</u>** have childbearing potential is defined as a female who is:
- Pre-menarche or post-menopausal for at least 1 year
- Surgically sterile
- Using an effective method of contraception from at least 4 weeks prior to the first vaccination until at least 10 weeks after the last vaccination (up to day 90). Effective contraception methods are described in the appropriate section.
- Has no heterosexual intercourses

[11] Such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)

	• Acute disease[12] at the time of vaccination • If there is a clinicalsuspicion of COVID-19 (according to the clinician's judgement), the clinical team will need to wait for the result of the PCR test for SARS-CoV-2, even if the rapid test is negative, and the vaccination will be delayed until the result comes back negative, the symptoms have resolved, and 4 weeks have elapsed since the first positive test result.

7.1.1 Effective Contraception for Female Volunteers

Female volunteers[13] are required to use an effective form of contraception during the course of the study. As this is a Phase I study, there is no information on the potential teratogenicity of the product. As there are no live viral components used in the composition or manufacture of the vaccine and thus no risk of sexual transmission of replicating viral products, males will not be asked to practice continuous contraception.

Acceptable forms of contraception include:

- Established use of oral, injected or implanted hormonal methods of contraception (for more than 1 month)
- Placement of an intrauterine device (IUD) or intrauterine system (IUS) (for more than 1 month)
- Barrier methods of contraception (condom or occlusive cap with spermicide)
- Male (partner) or female (self) sterilization.
- Abstinence of heterosexual intercourses

7.1.2 Influenza and SARS-CoV-2 vaccinations

Volunteers are required not to receive any vaccination during the 28-day period preceding or following either PepGNP-Covid19 injection (while for influenza, this period is reduced to 14 days preceding the IMP and 28 days afterward). Both SARS-CoV-2 and influenza vaccination is encouraged prior to the study

- >14-days before enrolment for Influenza and
- >28 days before enrolment for COVID-19

Since the study takes place during the influenza and SARS-CoV-2 vaccination period, an influenza vaccination will be proposed to the medical and paramedical staff during the 2-month pre-vaccination screening period (at least 14 days prior to experimental vaccination) or >28 days after second injection. Any such vaccination will be recorded in the participant's study record.

7.2 Recruitment and Screening

7.2.1 Recruitment

This study includes healthy adults aged 18-45 years residing in Switzerland.

Volunteers will be recruited from the local population around the study site in Switzerland, such as staff from the CHUV, Unisanté (who do not work in the department of the principal investigator), students, or any other interested individuals reached via public advertisements or information sessions. Such information sessions will be conducted at CHUV, Unisanté, UNIL, other health professionals, schools and directed at all staff and students in the context of general information about COVID-19.

Advertisements - formally approved by the competent ethics committee - will be disseminated via academic and hospital contacts as well as being posted in the following places:

- Intranet and social media platforms of the hospital and surrounding academic institutions (CHUV, Unisanté, UNIL, EPFL, nursing schools, Swiss TPH etc.)
- Intranet at the CHUV, Unisanté, HUG and Swiss TPH

[12] "Acute disease" is defined as the presence of a moderate or severe illness with or without fever according to investigator's judgment. All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. axillary temperature of ≤37.5°C.

[13] An individual who does **not** have childbearing potential is defined as a female who is:

- Pre-menarche or post-menopausal for at least 1 year
- Surgically sterile
- Using an effective method of contraception from at least 4 weeks prior to the first vaccination until at least 10 weeks after the last vaccination (until day 90). Effective contraception methods are described in the appropriate section.

- On stalls or stands at exhibitions or fairs in the surrounding hospitals (CHUV, Unisanté) and academic institutions (UNIL, EPFL, nursing schools, Swiss TPH)
- Via presentations (e.g. presentations at lectures or invited seminars)
- By email distribution to lists including staff, students and individuals who have already expressed an interest in taking part in any clinical trial at the CHUV or HUG

If the above do not provide sufficient participants, advertisements will be extended to

- Newspapers or other literature for circulation via press release
- Specific internet networks or agencies such as TrialReach

7.2.2 Screening (Day -60 to -1)

- **Informed consent.** All individuals showing interest in participating in the study will be provided with an information sheet (either by direct contact or electronically + phone call) and be given 72 hours to decide whether they would like further information from study investigator and/or sign the consent if willing.
- Informed consent will be taken before any screening procedure, as described previously in section 2.7. If consent is obtained, the screening procedures indicated in the schedule of attendances will be undertaken (**Table 2**).
- **Screening.** Consenting participants will undergo eligibility testing comprising of a structured interview on their medical history as well as a targeted physical exam. Blood and urine samples will also be collected for clinical laboratory tests which include general assessments of organ function[14] as well as screening for a panel of infectious diseases[15]. Specifically, all eligible participants will be screened for SARS-CoV-2 exposure just before vaccination.
    - **Physical exam.** BMI, pulse, blood pressure and temperature will be checked along with a general physical exam.
    - **Reacting to abnormal clinical or laboratory findings**. From the medical history, physical examination, urinalysis or blood tests at screening will be assessed as detailed in **Appendix B**. If an abnormal test result is deemed clinically significant it may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the investigator and within the inclusion and exclusion criteria.
    - **Pregnancy screening.** All eligible female participants will undergo a human chorionic gonadotropin β-subunit (βhCG) urine pregnancy test before receiving any vaccination (preformed again on the days of both proposed vaccinations to ensure the pregnancy status has not changed in the interim).
    - If the screening visit occurred more than 60 days before the vaccination visit as originally planned, a new medical history of the participant and a complete blood count (2.6 mL) will be carried out in the week preceding the vaccination (D-7 to D-1). Other laboratory tests may be realized if deemed necessary by a study physician according to potential medical events that may have occurred between screening and vaccination.

7.3 Assignment to study groups

7.3.1 Randomisation

A total of 26 eligible participants will be randomized into 1 of the following 4 groups:

- 10 Low Dose (LD) PepGNP-Covid19 (2.5 nmol)
- 3 Comparator (for LD)
- 10 High Dose (HD) PepGNP-Covid19 (7.5 nmol)
- 3 Comparator (for HD)

Each group will then be randomized into two sub-groups:

- A smaller "**pioneer**" group with 3 participants who will trial the first exposure to the dose
    - 2 vaccine vera

[14] Such as full blood count [FBC], alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin, serum creatinine Urine samples will be tested for the presence of protein, blood, and glucose
[15] Such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV)

  - 1 Comparator
- A larger "**follower**" group comprising the remaining 10 participants who will only be enrolled 5 days after the pioneer group if no holding rules were activated during this observation period.
  - 8 vaccine vera
  - 2 Comparator

Thus, 20/26 vaccine vera and 6/26 comparator controls.

The second "booster" vaccination will be identical to the first that the patient previously received.

Allocations of vaccine vera vs comparator for each group are double-blinded.

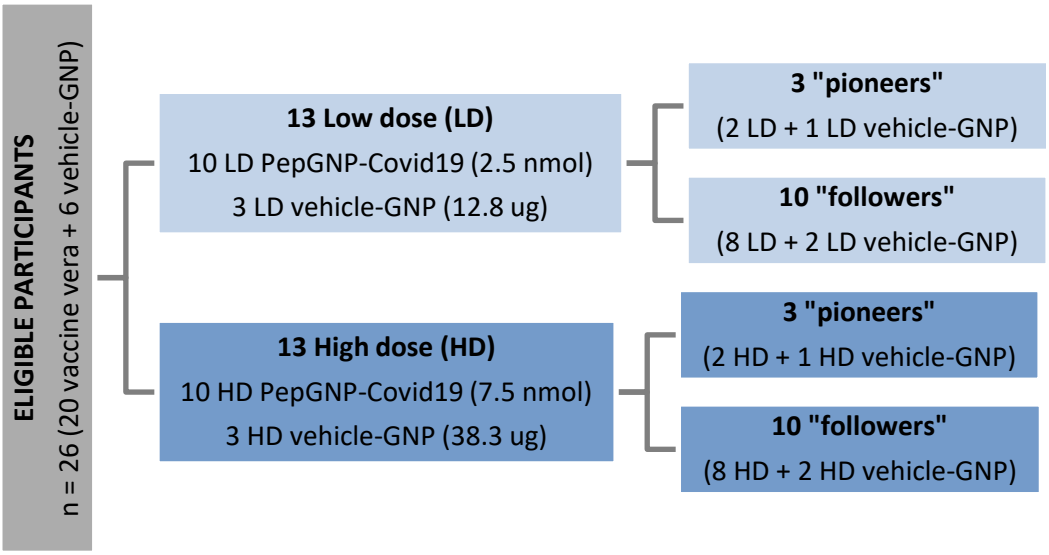
While the allocation of vaccine vera vs comparator will be double blinded and randomised, the allocation between LD or HD groups and the allocation to pioneer or follower groups will not be double blinded as the schedule reveals the allocation. Only the investigator would know this schedule, and so allocation of dosage (HD or LD) or allocation to the pioneer or follower group is single blinded to the volunteer.

Enrolment will follow a dose escalation strategy (LD--> HD) conditional to a Go/No Go DSMC review as depicted in **Figure 2**

A blinded randomisation list will be prepared and maintained by the pharmacy to assign groups and participants numbers at the clinical site. Thus, randomisation will be performed by the pharmacist.

Randomisation will only be performed once eligibility has been confirmed on Day 0.

The Pioneer group will be randomised by block of 3, while the 10 participants in the follower group will be randomised by block of 5.



7.4 Criteria for withdrawal / discontinuation of participants

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. The investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition, the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Volunteer non-compliance with study requirements.
- Any event which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.
- Recommendation by the Pharmacovigilance CRO or DSMC

The withdrawal category will be recorded in the case report form (CRF).

Any volunteer who fails to attend for three or more physical follow-up visits during the study without a clear rationale will be deemed to have withdrawn from the study.

7.4.1 Replacement of withdrawn participants

If a volunteer withdraws or is withdrawn before day 28 (or withdraws after day 28 but did not provide sufficient data or biological material to complete the analyses of the primary objective of the safety analysis) he/she will be replaced if possible, within the specified time frame (appropriate timing, vaccine dose available etc.).

In the event that the participant withdraws without providing sufficient data, after further recruitment is no longer possible, they will not be replaced and the statistical power to identify safety events will be adjusted in subsequent reports and publications.

8. STUDY INTERVENTION

8.1 Identity of Investigational Medicinal Products

8.1.1 Experimental intervention (PepGNP-Covid19)

The eight peptides selected from the COVID ligandome, are prepared as a quasi equimolar mix (i.e. almost 1:1:1:1:1:1:1:1). The concentration indicated for each dose group refers to the total concentration of all 8 peptides taken together with their GNP carrier molecules. The peptides are detailed in the Investigator Brochure.

Form Sterile aqueous, buffered solutions of PepGNP-Covid19 vaccine

Composition Each Low Dose (LD) 50 µl dose of peptide vaccine contains:

- 2.5 nmol of active substance (peptide)
- 12.8ug gold base particle
- Water For Injection (WFI)

Each High Dose (HD) 50 µl of peptide vaccine contains:

- 7.5 nmol of active substance (peptide)
- 38.3ug gold base particle
- Water For Injection (WFI)

8.1.2 Control intervention (Base particle Comparator, bpC)

Form The nano-shell vehicle is used for comparator

Composition Each Low Dose (LD) 50 µl of bpC contains:

- 12.8ug gold base particle
- Water For Injection (WFI)

Each High Dose (HD) 50 µl of bpC contains:

- 38.3ug gold base particle
- Water For Injection (WFI)

8.1.3 Packaging, Labelling and Supply

Vaccines will be diluted, labelled and dispensed to clinical team by the central Pharmacy at CHUV on the day of vaccination. This process entails reconstituting the freeze-dried powder with Water for Injection (WFI) with a volume stipulated in the IB. Once diluted, the vaccine/comparator is ready for drawing up in the syringe for administration.

The investigator or study nurse will collect the diluted product and draw up the required quantity for administration at the bedside.

Maintaining blinding during preparation: To maintain blinding and data protection, the pharmacist will not have access to the list linking the code to the volunteer identity. After reconstitution, there is no perceptible difference between prepared vials of vaccine vera and comparator and the volumes to be drawn into the syringe are also identical. The investigator will not be informed of the allocation.

8.1.4 Storage conditions

The vaccine and Base GNP will be stored in their freeze-dried form in a secure freezer at -20°C (temperature controlled) at the central pharmacy of CHUV.

Once reconstituted, the final product should be kept at room temperature.

8.2 Administration of interventions

The vaccine and Base GNP are to be injected using microneedles for intradermal injection into the deltoid region of the arm. The vaccine should not be administered intravascularly, subcutaneously or intramuscularly.

- Vaccinations will take place at the CTU CHUV-FBM in Lausanne where advanced life support drugs, resuscitation equipment and trained site staff will be immediately available for the possible management of anaphylaxis.

- When choosing an arm for the injection, clinicians should consider whether there is a local injury, skin issue or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection.
- If possible, the vaccines on day 0 and day 21 will be performed on different arms.

8.2.1 Experimental intervention (PepGNP-Covid19)

Two injections of 50 µl of PepGNP-Covid19 vaccine or comparator will be administered on days 0 and 21 using the Nanopass (described in **section 3.3.4**) microneedles for intradermal injection.

Briefly, the site will be cleaned with 70% alcohol and allowed to dry. A study nurse or investigator specifically trained in the use of the Nanopass needle will then inject the product intradermally. The development of a vaccinal weal will be noted along with any evidence of leakage.

A bandage will then be placed over the weal and the volunteer will be instructed to not touch the site for 1 hour, during which time they will be under medical surveillance.

At the end of the surveillance, the injection site will be inspected, and any abnormalities will be noted.

8.2.2 Control intervention (bpC)

As a double blind study, this will be identical to section 8.2.1.

8.3 Dose modifications

If dose modifications are recommended by the sponsor or DSMC, their use will be included in an amended version of the protocol subject to approval by the CEC and Swissmedic before implementation.

8.4 Compliance with study intervention

All doses in this vaccine study will be administered by the investigational team and recorded in the eCRF. The study medication will be at no time in the possession of the volunteer and compliance to dosage will not, therefore, be an issue.

Non-compliance is defined as missing >50% of physical exams or daily diary entries. These participants will still be considered in the ITT analysis.

Any volunteer who fails to attend the booster vaccination or has other significant protocol deviations, will be considered in the intention-to-treat analyses where appropriate.

8.5 Data Collection and Follow-up for withdrawn participants

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored. All safety and immunogenicity data collected from a volunteer that withdraws after vaccination, or is withdrawn by the investigator, will be used in the per-protocol analysis (if time-point is before withdrawal), or in the intention-to-treat analysis (if time-point is after withdrawal). The blinded allocation of the withdrawn participant will only be revealed at the end of the trial on an opt-in basis.

8.6 Trial specific preventive measures

8.6.1 Medications

- No specific restrictions on medications or treatments are imposed, however, all medications taken during the trial will be logged at each contact in the eCRF.
- In case of fever or pain, paracetamol is recommended (preferred over NSAID) if the patient feels such an intervention is required.

8.6.2 Contraception

The method of contraception for female participants is at the choice of the participant and no specific methods are contraindicated or favoured.

8.6.3 Vaccines

Vaccinations are part of the exclusion criteria and are not permitted until 4 weeks after the 2nd vaccination

unless a medical necessity. Any such vaccination will be recorded in the participant's study record.

8.7 Concomitant Interventions (treatments)

- No specific restrictions on routine/concomitant medications or treatments are imposed, however, all medications taken during the trial will be logged at each contact in the eCRF.
- Also, significant deviations in routine/concomitant medication will also be logged.

8.8 Vaccine Accountability

The supply, storage, distribution, return and potential destruction of the vaccines will be undertaken by CHUV pharmacy using GLP SOPs (including recording reception date, batch, expiration date, quantity received, shipment, date of dispensation, participant ID, quantity dispensed, quarantine and destruction or return of unused vials).

8.9 Return or Destruction of Study Drug

Unused vaccine and base particle comparator will be destroyed according to GLP by CHUV pharmacy.

9. STUDY ASSESSMENTS

9.1 Table of study procedures and assessments

See next page

9.2 Assessments of outcomes

Please see section 5.

Table 3: Schedule of study procedures

Visits	1 Screening	2 Vaccination 1	3	4	5	6 Vaccination 2	7	8	9	10	11	12
Timeline (days)	Any time from -60 to -1	0	1	7	14	21	Day 1 after vaccination 2	Day 7 after vaccination 2	35	60	90	180
Tolerance (days)		0	±1	±1	±2	±2	±1	±1	±2	±7	±14	±14
Summary of interventions												
Screening and trial procedures												
1. Inclusion/Exclusion criteria	✓	✓ *(confirmation)*				✓						
2. Informed consent	✓	✓ *(confirmation)*										
3. Medical history	✓	✓ *(confirmation)*				✓				✓	✓	✓
4. Concomitant medication	✓	✓ *(confirmation)*	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5. Physical exam	✓	✓ *(confirmation)*	(✓)	(✓)	(✓)	✓	(✓)	(✓)	(✓)	(✓)	(✓)	(✓)
6. Compensation (CHF)	0	100	0	100	100	100	0	100	100	0	100	100
Intervention												
7. Study Vaccination		✓				✓						
Safety monitoring *(Safety monitoring is continuous. Opt-in reporting is active on all days throughout study)*												
8. Provide diary card		✓(1st)				✓(2nd)						
9. Collect diary card + photo (if any)					✓(1st)				✓(2nd)			
10. Solicited local reactogenicity		✓	✓	✓		✓	✓	✓				
11. Solicited systemic reactogenicity		✓	✓	✓	✓	✓	✓	✓	✓			
12. Review AE/SAE/AESI		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Samples taken for blood safety tests and serology												
13. Complete blood count, 2,6 mL EDTA	2,6 ml			2.6 ml	2.6 ml			2.6 ml	2.6 ml			
14. Biochemistry, 2.6 mL LiHe	2.6 ml			2.6 ml	2.6 ml			2.6 ml	2.6 ml			
15. HIV, HBV, HCV, 4.9 mL EDTA	4.9 ml											
16. SARS-CoV2 test (PCR/antigenic test)		✓				✓						
17. βHCG, urine test	✓ (urine)	✓ *(pre-vaccine)*				✓ *(pre-vaccine)*						
18. Contraception	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
19. Urinary protein, blood, glucose	✓ (urine)											
20. Anti-nuclear antibodies[a] 7.5 mL serum gel	7.5 ml										7.5 ml	

Samples taken for secondary immunogenicity studies												
21. HLA typing, 4.9 mL EDTA		4.9 ml										
22. PBMC & plasma, 7.5 mL LiHe		67.5 ml *(pre-vaccine)*				52.5 ml *(pre-vaccine)*			67.5 ml		52.5 ml	67.5 ml
23. Antibody tests, 2x4.9 ml serum-gel		9.8 ml *(pre-vaccine)*				9.8 ml *(pre-vaccine)*			9.8 ml		9.8 ml	9.8 ml
Secondary Immunogenicity Outcomes												
24. Anti-SARS-CoV-2 IgG response		✓				✓			✓		✓	✓
25. CD8 T cell response [b]		✓				✓			✓		✓	✓
Exploratory Immunogenicity Outcomes												
26. Ig functional assay [c]		(✓)				(✓)			(✓)		(✓)	(✓)
27. T and B cell responses assessment [d]		(✓)				(✓)			(✓)		(✓)	(✓)
TOTALS												
Summary of interventions												
Daily vol (ml)	17.6	82.2	0.0	5.2	5.2	62.3	0.0	5.2	82.5	0.0	69.8	77.3
Max cumulative vol (ml)	17.6	99.8	99.8	105.0	110.2	172.5	172.5	177.7	260.2	260.2	330	407.3
Compensation cumulative (CHF)	0	100	100	200	300	400	400	500	600	600	700	800

Legend for Table 2

[a] As a surrogate for autoimmune reactions. A 4-fold increase from baseline along with a positive history of clinical signs/symptoms in line with autoimmunity, will be investigated as an adverse event.

[b] Frequency of peptide-specific CD8+ T cells on PBMCs by 2 flow cytometry methodologies: ex vivo, using staining with vaccine specific dextramers, CD3, CD4, CD8 and memory markers (CD45RA, CCR7) at D0, D35 and D180; and using activation-induced markers (AIM) CD69, CD137 and CD107a, in response to peptide stimulation *in vitro*, at D0, D21, D35, D90 and D180.

[c] Serum Ig functional assay, neutralization and enhancement, performed if the vaccine induces anti-SARS-CoV-2 antibodies, as assessed by anti-SARS-CoV-2 serology (24). .

[d] Additional exploratory analyses of Coronavirus-specific cellular responses at various time-points: Additional characterization of the vaccine induced response, cytokine secretion, Coronavirus-specific cell functional assays.

Physical consultation at study site	✓ Performed
Blood draw	(✓) Performed if deemed necessary
	+photo if any In the first 7 days post-vaccination participant will be asked to take a photo of the injection site in the event of signs/symptoms of local reactogenicity
Vaccination	
Telephonic consultation	

9.3 Procedures at each visit

9.3.1 Intervention (Day 0 and 21)

- **Pre-vaccine baselines.**
  - Before vaccination, a baseline blood sample will be taken (analyses summarised in **Table 2**)
  - Additionally, medical history and eligibility criteria will be confirmed in case of changes from screening).
  - Volunteers will not be considered enrolled in the study until they have received the first vaccine.
- **Vaccination.** Two intradermal injections of the IMP or control will be administered in the upper arm of each consenting participant. The injections will take place on days 0 and 21 using the Nanopass MicronJet600 (https://www.nanopass.com/product/) microneedle.

  Risk will be minimised by:
  - **Dose escalation strategy (LD--> HD)**
    - Escalation from low to high dose enrolment is conditional to a Go/No Go DSMC review as depicted in **Figure 2**.
  - **Staggering enrolment**
    - Enrolment in either LD or HD will be split into a smaller "pioneer" group of 3 participants (2 vaccine vera) and a larger "follower" group (8 vaccine vera).
    - There will always be at least 24 hours between successive vaccinations in the pioneer group.
    - In the follower groups, up to 5 vaccinations can occur in a single 24h period with a period of at least an hour between each.
  - **Immediate post-vaccinal monitoring**
    - The participant will be monitored for 60 min after vaccination for adverse reactions.
    - Briefly, directly after vaccination, the injection site will be covered with a dressing and the volunteer will stay in the clinical unit for observation in case of immediate adverse events. Observations will be taken during the 60 minutes following vaccination (+/- 5 minutes). The dressing will then be removed, and the injection site inspected before the volunteer leaves.
    - Advanced Life Support drugs resuscitation equipment and trained site staff will be immediately available for the possible management of anaphylaxis
- **Preparation for follow-up.** A thermometer, ruler, diary card and participant identification card will be given to each volunteer, with instructions on use, along with the 24-hour emergency telephone number to contact the on-call study physician if needed.

9.3.2 Follow-up (Day 0 to 180)

- **Contacts**
  - **Days 0-14 and Days 21 to 35.** A participant diary will be filled out daily for 14 days after each vaccination from day 0 and day 21 to capture adverse events, body temperature and concomitant medication.
  - In the event of local reactogenicity (pain/swelling/redness at injection site), the participant will be instructed to note all symptoms in the provided diary and take a photo of the injection site with the provided ruler placed on adjacent skin in the photographed frame. The patient is instructed to not include any identifying features in this photograph such as his/her face. This photo will be provided to investigators in the following visit and stored in the CHUV secured server.
  - **Day 1 and Day 22.** The participant will be called 1 day after each vaccination to screen for adverse events (the presence of adverse events or patient's concerns may trigger a physical examination).
  - **Days 7, 14, 28, 35, 90 and 180.** Six further physical consultations with blood draws will then take place over a 180-day (6-month) follow-up period.
  - **Day 60.** The participant will be called on day 60 to screen for adverse events (the presence of adverse events may trigger a physical examination).

9.3.3 Analyses (during and after the end of recruitment)

Blood and urine samples will be processed at the following sites:

1. At the CHUV haematology, immunology and biochemistry laboratories, using standard procedures (all participants):
   - **Haematology:** Full Blood Count (Hb, Ht, RBC, MCV, platelets, WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils)
   - **Biochemistry:** Creatinine, AST, ALT, total bilirubin, γ-GT, alkaline phosphatase
   - **Diagnostic serology:** HBsAg, HBsAb, HBcAb, HCV antibodies, HIV antibodies
   - **Urinalysis:** Urine will be tested for protein, blood and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening and immediately prior to the vaccinations (Day 0 if >7 days from screening and Day 21).
   - **Immunology**: Human Leukocyte Antigen (HLA) typing

2. At the CHUV immunology research laboratory and/or collaborating laboratories:
   Secondary outcomes:
   - Anti-SARS-CoV-2 antibody
   - CD8 T cell responses by cytometry (dextramer staining; AIM (activation induced marker)).

   Exploratory analysis:
   - Additional characterization of the responses observed: phenotyping, cytokine secretion in response to vaccine peptides.

   Additional exploratory analyses (in line with the aims of the protocol) of immunological response to COVID-19 vaccine such as functional assays may be performed at the discretion of the investigators and Sponsor on a subset of selected samples.

   Handling possible suspected natural SARS-CoV-2 infections
   - All fevers or flu like illnesses will be assessed for COVID-19. SARS-CoV-2 antibody titres will also be taken before, during and after the study.
   - This includes any instance of fever ≧37.5°C

3. Sample repository is located at the CHUV immunology research laboratory. Samples will be frozen in liquid nitrogen and kept for 10 years. Collaboration with other specialist laboratories in Switzerland, Europe and outside of Europe for further exploratory laboratory tests related to the trial may occur. This would involve the transfer of serum or plasma and/or PBMC to perform analysis to these laboratories, but the samples would be identified only by a code number. The laboratory tests will be conducted according to the procedures established in the test laboratories.

10. SAFETY

The assessment of safety is the primary outcome of this study.

During the entire duration[16] of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in participant diaries and case report forms (CRF).

The Sponsor's WIs provide more detail on safety reporting.

10.1 Definition and assessment of adverse and other safety related events

10.1.1 Definition of Adverse events (AEs)

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered causally related to the study intervention.

The following guidelines will describe how an AE is recorded/categorised in the study/safety database:

10.1.1.1 Solicited AEs (i.e. reactogenicity parameters)

Solicited AEs will be recorded by the patient on diary cards daily for 14 days after each vaccination (7 days for local AEs and 14 days for systemic AEs). The diary cards will be collected and entered into the eCRF 14 days after each vaccination.

- A consensus list of recognized solicited local and systemic AEs is recommended in the *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* document produced by the U.S. Department of Health and Human Services Food and Drug Administration (FDA) in 2007[17]
- These recommendations have been adapted and complemented for this trial and summarised in the tables presented in **Appendix A** (solicited local and systemic clinical measures)

10.1.1.2 Unsolicited AEs

Unsolicited AEs are adverse events that do not appear in the list of solicited AEs.

Unsolicited AEs of all severities will be recorded throughout the study period and will be entered in the study database (eCRF) using the corresponding "Preferred term" listed in the CTCAE [18]. The safety database managed by the Pharmacovigilance (PV) CRO will also capture the MedDRA terminology to ensure interoperability across studies.

10.1.1.3 Adverse events of special interest (AESI)

Adverse events of special interest will be recorded at each contact.
The list of AEs considered of special interest for both naNO-DENGUE and naNO-COVID constructs is adapted from the *D2.3 Priority List of Adverse Events of Special Interest: COVID-19* document, published by the SPEAC (Safety Platform for Emergency vACcines) in 2020 [19]. AESI will be captured using the CTCAE terminology.
AESI include (exhaustive list):

- Generalized convulsion
- Guillain-Barré Syndrome (GBS)
- Acute disseminated encephalomyelitis (ADEM)
- Thrombocytopenia
- Anaphylaxis
- Vasculitides
- AE grade 3

[16] Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period (180 days in total).
[17] Available here: https://www.fda.gov/media/73679/download
[18] Available here: here: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf
[19] Available here: https://brightoncollaboration.us/wp-content/uploads/2020/06/SPEAC_D2.3_V2.0_COVID-19_20200525_public.pdf

10.1.1.4 Serious Adverse events (SAEs)

A SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death
- Life-threatening event (i.e. the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred).
    - This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation [20], regardless of length of stay, even if it is a precautionary measure for continued observation, or prolongation of existing hospitalisation.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgement, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above.
    - Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.
- Congenital anomaly or birth defect [21].

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease.

Should the investigator become aware of any SAEs experienced outside of the study follow up period that he/she thinks may be related to the IMP these will be reported to the pharmacovigilance support entity (Sponsor/CRO).

10.1.1.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)

- The investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR.
- All SUSARS will be unblinded by the PI in order for their assessment. This assessment will be undertaken by the DSMC.

10.1.1.6 Safety signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures.

10.1.1.7 Safety holding rules

The safety holding rules which will result in an immediate suspension of the trial (where restart is subject to DSMC review and CEC approval) are listed below:

Table 4: Safety holding rules

Solicited local or systemic adverse events of grade 3	> 34% of volunteers[22] within any group or subgroup develop a Grade 3 solicited local or systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >48 hrs.
Unsolicited adverse events	> 34% of volunteers[26] within any group or subgroup develop a Grade 3 unsolicited adverse event (including a laboratory adverse event) that is considered possibly, probably or definitely related to either vaccination and persists at Grade 3 for > 48hrs.
SAE	> 34% of volunteers[26] within any group or subgroup develop an SAE
SUSAR	A SUSAR related to the investigational peptide vaccination occurs

[20] **Note:** Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly **does not** constitute a serious AE (SAE).
[21] **Note:** rigorous testing, counselling and medical history taking will work to best ensure than pregnancies do not occur within the timeframe of this study.
[22] More than 34% of the 3 members of the pioneer subgroups is ≥ 2/3. More than 34% of the 13 members of the entire dosage group is ≥ 4/13.

Death	Death related to the investigational peptide vaccination occurs

10.1.2 Assessment of AE

10.1.2.1 AE Causality

All AE will be evaluated for causality by the investigational team. In addition, Sponsor (through delegated PV CRO) make a causality assessment of all SAE.

Table 5 below is an adaptation of the ICH E2A guidelines providing more detail on the decision logic for assessing causality. Related AEs are graded as "*Possible"*, "*Probable"* or "*Definite".*

Table 5. *Guidelines for assessing the relationship of vaccine administration to an AE.*

Not related	**No Relationship**	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
Related	**Possible**	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
	Probable	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
	Definite	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

10.1.2.2 AE severity

10.1.2.2.1 Severity of solicited AE

- Severity of solicited AE is graded according to an adapted version of *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* document, produced by the U.S. Department of Health and Human Services Food and Drug Administration (FDA) in 2007[23].
- The slight adaptation of these guidelines is that we will not be using "grade 4" severity (as grade 3 is considered a stopping rule).
- These recommendations are summarised in the tables presented in **Appendix A** (Solicited local and systemic AEs).

10.1.2.2.2 Severity of unsolicited AE and AESI

- Severity of Unsolicited AEs is graded according to the CTCAE[24] by the investigator.
- For laboratory measures, a modified version of the *Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* document and CTCAE will be used for severity grading as summarized in **Appendix B** (Unsolicited abnormal laboratory measures).

[23] Available here: https://www.fda.gov/media/73679/download
[24] Available here: here: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

10.1.3 Follow up and actions taken in response to AEs

10.1.3.1 Follow up

- All non-serious AEs related with the IMP (up to and including grade 2) will be followed until resolution/stabilisation.
- AEs of grade 3, AESI, SAEs, SUSARs or any AE that results in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution, or until a non-study related causality is assigned (if the volunteer consents to this).

10.1.3.2 Actions taken for individual participants

All clinically relevant AEs will be monitored and handled according to the medical judgement of the clinical investigator.

The volunteer may be followed up more intensively for the purposes of monitoring the evolution of the AE. For example, a telephonic contact may be transformed into a physical visit so that a clinical exam or further blood samples and investigations relevant for the resolution of the AE may be undertaken. Additionally, the volunteer may be referred for specialist assessment.

All actions will be logged and incorporated into the assessment of AE severity.

10.1.3.2.1 Abnormal laboratory measures

All clinically relevant deviations on laboratory measures will be investigated according to the medical judgement of the clinical-investigator.

10.1.3.3 Actions taken on all participants

10.1.3.3.1 Safety Holding Rules

If a holding rule is activated, then all further vaccinations will be held and the allocation of the relevant participants or (as a second step and only if needed) the whole dose group will be unblinded.

10.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting rules are summarised in **Figure 5** below.

All AEs (occurring at any point in the trial) that are observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be documented in the eCRF.

Outside of the reporting deadlines specified below (for SAEs, SUSARs, Safety Signals relevant to holding rules), all AEs will be transmitted to the Sponsor through a delegated Pharmacovigilance CRO (PV CRO) at regular intervals (aligned with steps 1a, 1b, 1c, and 1d in **Figure 2** as well as at the end of the study).

10.2.1 Reporting of SAEs

Note that the * indicates "since investigator is first made aware of the safety event in question".

- **PI → Sponsor/PV CRO (24h*):** The Principal investigator (or any delegated site staff) will report immediately and within 24 hours all SAE to the PV CRO. SAEs will be reported by email via the REDCap survey tool). All elements captured within the SAE form featured in the eCRF will be transmitted. This includes free text and auxiliary information required for appropriate assessment of the severity and causality of the SAE. A separate email containing relevant coded source documents (laboratory or radiological exams) will be sent simultaneously.
- **Sponsor/PV CRO → PI (48h*):** The CRO will re-evaluate the SAE and send clarifications/approval to the study team within 48 hours by email.
- **Sponsor/PV CRO → DSMC (72h*):** The DSMC will be informed within 72h of the SAE by the Sponsor. The communication will be generated by the PV CRO via email.
- *If fatal:* **PI → CEC (7d*):** SAEs resulting in death are reported to the Ethics Committee via BASEC within 7 days by the principal investigator.
- *If not fatal:* **No further expedited reporting action on initial event.**
  - Periodic follow up on the SAE is then reported to the PV CRO follow-up on new relevant information/issue modifications via the REDCap survey forms as before.
  - The SAE will be followed until a satisfactory resolution/stabilisation occurs.

- The non-fatal SAEs will be submitted to CEC in the annual safety report.

10.2.2 Reporting of SUSARs

- **PI → Sponsor/PV CRO (24h*):** The Principal investigator or any delegated site staff will report immediately and within 24 hours all SUSARs to the PV CRO. SUSARs will be reported by email via the REDCap survey tool. All elements captured within the SAE form featured in the eCRF will be transmitted. This includes free text and auxiliary information required for appropriate assessment of the severity and causality of the SUSAR. A separate email containing relevant coded source documents (laboratory or radiological exams) will be sent simultaneously.
- **Sponsor/PV CRO → PI (48h*):** The CRO will re-evaluate the SUSAR and send clarifications/approval to the study team within 48 hours. Once validated, a CIOMS form will be generated (within 4 days of the event, at the latest). and communicated to PI for reporting to EC
- **Sponsor/PV CRO → DSMC (72h*):** The DSMC will be informed by email within 72h of the SUSAR by the Sponsor.
- *If fatal:*
  - **PI → CEC (7d*):** SUSARs resulting in death are reported to the Ethics Committee via BASEC within 7 days by the principal investigator (using the CIOMS form produced by the PV CRO).
  - **Sponsor/PV CRO → Swissmedic (7d*):** SUSARs resulting in death are reported to Swissmedic via email within 7 days by the Sponsor/PV CRO (using the CIOMS form produced by the PV CRO).
- *If not fatal:*
  - **PI → CEC (15d):** SUSARs not resulting in death are reported to the Ethics Committee via BASEC within 15 days by the principal investigator (using the CIOMS form produced by the PV CRO).
  - **Sponsor/CRO → Swissmedic (15d*):** SUSARs not resulting in death are reported to Swissmedic via email within 15 days by the Sponsor/CRO (using the CIOMS form produced by the PV CRO).
    - Periodic follow up on the SUSAR is then reported to the PV CRO
    - Follow-up information is captured in the REDCap survey forms as before.
    - The SUSAR will be followed until a satisfactory resolution/stabilisation occurs, or until a non-study related causality is assigned (if the volunteer consents to this).

10.2.3 Verification and Reporting of holding rule AEs and AESIs

See **Table 4** for holding rules

- **PI → Sponsor/PV CRO (24h*):** In addition to SAE and SUSAR, the Principal investigator (or any delegated site staff) will immediately report and within 24 hours all AESIs to the PV CRO as delegated by the Sponsor. This reporting will allow the PV CRO to actively screen for activation of one of the safety holding rules. All elements captured within the AE form featured in the eCRF will be transmitted. This includes free text and auxiliary information required for appropriate assessment of the severity and causality of the AE. A separate email containing relevant coded source documents (laboratory or radiological exams) will be sent simultaneously.
- **Sponsor/PV CRO → PI and DSMC (72h*):** The PV CRO will continually monitor the reported holding rule AEs and inform the PI and DSMC as soon as a holding rule is met via email (**Table 4**).
- *If it results in the activation of a holding rule:*
  - **PI → CEC (7d):** the holding rule will be reported to the Ethics Committee via BASEC within 7 days by the principal investigator.
  - **Sponsor/PV CRO → Swissmedic (7d*):** the holding rule will be reported to Swissmedic via email within 7 days by the Sponsor/PV CRO.

10.2.3.1 Reporting activation of holding rules

- In the case a holding rule is met, staff will be immediately informed, and the trial halted.
- If a holding rule is activated, then all further vaccinations will be held and the allocation of the relevant participants or the whole dose group (as a second step and if necessary for evaluation) will be unblinded.

10.2.3.2 Reporting trial termination/suspension

- If the trial is prematurely terminated or suspended, the PI will promptly inform the CEC and Swissmedic of the reason for termination or suspension.

- If the trial is prematurely terminated for any reason, the PI will promptly inform the trial participants and should assure appropriate care and follow-up.

10.2.4 Reporting of safety signals

All suspected new risks that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor within 24 hours. The Sponsor must report the safety signals within 7 days to the Ethics Committee via BASEC and to Swissmedic.

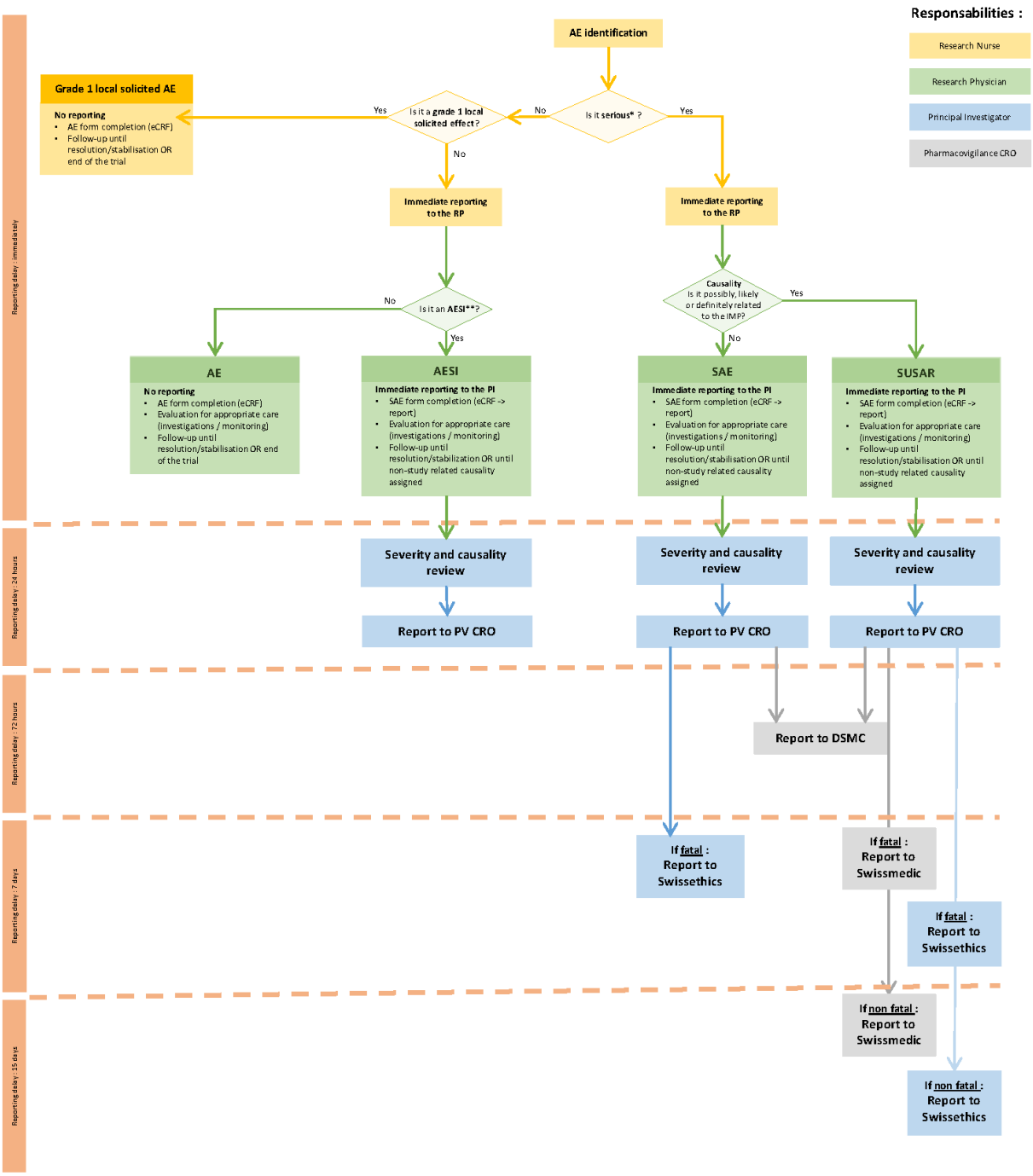
10.2.5 Reporting and Handling of Pregnancies

Pregnant participants will immediately be withdrawn from the clinical study. Any pregnancy during 180 days of the trial will be reported to the Sponsor/PV CRO within 24 hours through dedicated form in REDCap sent by email through survey tool. The course and outcome of the pregnancy will be followed up carefully until outcome, and any abnormal outcome regarding the mother or the child will be documented and reported.

Follow-up for pregnancy and the new-born will be assessed on a case-by case basis by specialist. At a minimum this will entail documenting and reporting routine visits and pregnancy outcomes until the end of the neonatal period.

10.2.6 Assessment, notification and reporting on the use of radiation sources

No radiation exposure is anticipated in this study.



*An AE is serious when it results in any of the following outcomes, whether or not considered related to the study intervention :

- Death
- Life-threatening event
- Persistent or significant disability or incapacity
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation, or prolongation of existing hospitalisation.
- An important medical event that may jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect

** An AESI is one of the following AE :

- Generalized convulsion
- Guillain-Barré Syndrome (GBS)
- Acute disseminated encephalomyelitis (ADEM)
- Thrombocytopenia
- Anaphylaxis
- Vasculitides
- AE grade 3

Figure 5 Reporting rules for safety events

10.3 Safety reviews

See **Figure 2** for overview.

Safety reviews will consider the following:

- The relationship of eventual AE or SAE to the vaccine.
- The relationship of eventual AE or SAE to the vaccine *dose*, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms, and alterations to the current Participant Information Sheet are discussed.
- Eventual new, relevant safety information from ongoing research programs on the various components of the vaccine (i.e. the peptides and the nanoparticle).

Apart from planned analyses, safety data will be continuously monitored. Adverse events will be reviewed by the PI and PV CRO as they arise and according to the above reporting guidelines. The Sponsor will be consulted at each decisional checkpoint.

10.3.1 Data and Safety Monitoring Committee (DSMC) reviews

A Data and Safety Monitoring Committee (DSMC) will be established prior to the trial initiation and will include at least two independent clinicians and one epidemiologist/statistician with relevant expertise in the field or in vaccine development and evaluation of vaccine safety.

The naNO-COVID trial is initiated after an interim analysis of safety data from the naNO-DENGUE trial (master protocol). The naNO-COVID construct is created with identical nano technology and differs only in the conjugated peptide cocktail, and thus serves as an adequate preliminary safety assessment of the base particle comparator.

Planned interim analysis

An interim analysis by the DSMC will take place using a minimum of 7 days of follow-up data on at least 10/13 participants from the low dose naNO-COVID group.

If no holding rules are activated (see below) the DMSC will review the merits for

1) Escalating the dose of naNO-COVID from LD→ HD (i.e. enrolling 3 pioneer HD participants)

Proceeding with the booster vaccine

Unplanned reviews

The DSMC will also review any SAEsor as soon as a holding rule is activated.

The DSMC may also be contacted for advice and independent review by the Investigator or trial Sponsor for any other situation where the Investigator or trial Sponsor feels independent advice or review is important or necessary.

10.3.2 PV CRO Safety Reviews

The safety profile of the investigational product will be assessed on an ongoing basis by the investigators with 2 planned internal safety reviews performed by the PV CRO.

These reviews will serve as Go/No Go checkpoints to continue enrolment within a dose group once a small “pioneer” subset of three individuals have been vaccinated and observed for at least 24h. This serves to minimize risk exposure and initiate early safety responses.

An annual safety report is submitted once a year to the CEC via the Principal Investigator and to Swissmedic via the PV CRO/Sponsor.

11. STATISTICAL METHODS

As this study has no statistical hypothesis test, there is no formal power calculation.

Since this is a first-in-human study with focus on safety, the number of participants exposed to test products needs to be limited. Due to this limitation, only adverse events with high incidence rate will be detected as summarized in the table below.

11.1 Determination of Sample Size

Having ten participants per group (with 20 exposed to the investigational product at either dose), would allow 80% power of detecting an AE with a true incidence of:

- 5% across all exposed participants (LD and HD combined) or
- 20% within a single dose group (LD or HD)

Achievable statistical power (1-β) to observe at least 1 AE at various incidences (λ) within the investigational sample size

True incidence of the AE (λ)	Sample size required to detect a single AE at the statistical power (1-β) listed below		
	50%	80%	95%
2.5%	28	64.4	120
5%	14	32.2	60
10%	7	16.1	30
20%	3.5	8.05	15
30%	1.75	4.03	7.5

KEY

At least a single ***dose-dependent*** AE would be detectable at the given probability within a single dose group of 10 participants (either LD or HD)
At least a single ***exposure-dependent*** AE would be detectable at the given probability across all dose groups of 20 participants
AE not detectable at this probability within the investigational sample size

Thus, the proposed sample size provides reasonable confidence to assess safety of the novel peptide vaccine and is in line with similar studies previously approved for Phase I evaluation, and at the same time efficiently assessing the evidence of an immunologic response as a surrogate of protection against severe COVID-19 disease.

11.2 Safety analysis

Safety analysis will be carried out for all vaccinated participants, regardless of whether or not they complete the study.

Analysis of the data from this study will be descriptive in nature and no formal statistical hypotheses will be tested. Mean, standard deviation, minimum, maximum (possibly median and quartiles) will be used for continuous variables and number and percentage will be used for categorical variables, unless otherwise specified in the protocol.

Analysis will be applied on per-protocol population, thus only those participants who receive vaccination will be included in the analysis.

The following statistical analyses will be performed on safety data:

1. An external DSMC-led review on a minimum of 24h follow-up data the first 13 participants in the LD group (following the first of two planned vaccinal doses).
2. A final analysis on 6-month follow-up data of all 26 patients spanning all vaccinal doses.

11.3 Statistical Methods for Primary Endpoints:

Event analyses will include the following

- Occurrence of each solicited local adverse event within a 7-day follow-up period (day of each vaccination and 7 subsequent days) after each vaccination.
- Occurrence of each solicited systemic adverse event within a 14-day follow-up period (day of vaccination and 14 subsequent days) after each vaccination.

- Occurrence of unsolicited adverse events within 21 days (day of vaccination and 21 subsequent days) after each vaccination[25].
- Occurrence of a serious adverse event from the first vaccination to the end of the study.
- Solicited and unsolicited AE data will be collected at each clinic visit. It will be collected from diary cards, clinical review, clinical examination (including observations) and laboratory results. This AE data will be tabulated, and frequency, duration and severity of AEs compared between groups.
- All SAEs will be reported. SAEs, AEs of special interest and withdrawal due to AE(s)/SAE(s) will be described in detail, and relatedness to vaccine will be assessed.
- Haematological and biochemical laboratory values will be presented according to toxicity grading scales and tabulated by group (**Appendix B**).
- The incidence, intensity, and relationship of individual solicited and unsolicited AEs to the vaccine administration will be calculated overall and by group.
- Presentations will include the number and percentage of participants with at least one solicited symptom (local or systemic), at least one local symptom, and at least one systemic symptom, as well as the incidence of each symptom individually.
- The number of participants with at least one report of an unsolicited adverse event reported up to 21 days after the vaccine will also be summarized overall and by group.

11.4 Immunogenicity analysis

For secondary immunogenicity endpoints, descriptive summaries and plots over the time course for both individual volunteer results and groups will be presented. Where appropriate, highly skewed data will be log-transformed and presented as geometric means with confidence intervals. The statistical analysis for immunogenicity will be based on both intention-to-treat and per-protocol principle. Response to vaccination will be evaluated by intra-group comparison of post / pre vaccination results. Assessment of optimal vaccine dose will be evaluated by inter-group comparisons of responses at various time-points.

[25] *The occurrence of unsolicited adverse events will in fact be monitored until the end of the study, but only those events occurring within 21 days after the vaccination will be used for the analysis of this primary safety endpoint.*

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

12.1.1 Case Report Forms

All protocol-required information will be entered in electronic CRFs by the Principal Investigator (or delegated site staff). All source data (such as patient diaries, laboratory results etc.) and volunteer CRFs will be stored securely.

Only the Sponsor, PIs, and delegated site staff will be allowed to access the eCRFs.

The Sponsor will have a limited view of the eCRFs that contain strictly no identifying information.

Once identifying information is no longer required for the basic functioning of the trial (i.e. the requirement to identify patient, address and telephone for follow-up interviews), all eCRFs will be strictly coded (removing the name, initials address and birth date) and only the participant number in combination with year of birth will remain.

This identifying information that is removed from eCRFs at the end of participant follow-up will be transferred to a separate secured file which is independent from the eCRFs. And this will be made available only under request to the PI (if the request pertains to pharmacovigilance monitoring, quality assurance reviews, audits and evaluation of the study safety or a medical concern of the patient, with the patients' consent).

Only the Sponsor representatives, Investigators, the clinical monitor, the CEC and regulatory authorities will have access to the records.

Once the follow-up period of a patient is terminated, all data will be coded: volunteer data will be identified by a unique study number in the eCRF and database.

The study identifier is a unique 3 digit number, automatically generated by REDCap, preceded by the letter "C" (for naNO-COVID). A -LD or -HD suffix is then added to specify whether the participant belongs to the low dose or high dose group (e.g.: C-011-LD).

A separate independent confidential file containing identifiable information will be stored in a secured location in accordance with data protection requirements.

12.1.2 Specification of source documents

Source documents are original documents, data, and records from which the volunteer's CRF data are obtained. For this study, medical notes (medical history, vital signs, physical examination, adverse event data, concomitant medication and randomisation number) collected at each visit will be directly entered in electronic CRF. Patient diaries, laboratory records and any additional relevant medical information (e.g. emergency department records and complementary exams in case of serious adverse event), will be collected and stored in paper format. Source data will be secured in Unisanté clinical archives with access granted only to medical professionals participating in the trial.

12.1.3 Record keeping / archiving

The Investigators will maintain appropriate medical and research records for this trial (minimum 10 years), in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The PI, co-Investigators and clinical research nurses, and medical professionals will have access to records. The Investigators will permit authorized representatives of the Sponsor, monitors, as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of monitoring, quality assurance reviews, audits and evaluation of the study safety and progress.

All the study documents will be kept at site in an Investigator Site File (ISF) while the Trial Master File (TMF) will be kept up to date by Sponsor. All the regulatory submission documents to Swissmedic will be kept in a country specific TMF at site and transferred to Sponsor at the end of the study.

12.2 Data management

The Principal Investigator will have the responsibility for overseeing the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study by designated persons. Data management will be performed by Unisanté. Throughout, regular data collection and monitoring, clinical data reported on CRFs and/or relevant serological/biological samples analysis results scheduled in the protocol will be integrated into the electronic Data Capture System (REDcap database). This includes safety data, safety laboratory data and

outcome data. Immunological data will be kept in a separate file with the same level of security. The Safety Database is “AB Cube SafetyEasy PV“ which is hosted on AB Cube servers within the EU. AB Cube’s data centres are located at two sites in France, one site holds the primary database servers and the other holds back-up servers for disaster recovery purposes.

For each batch of data, quality control and triggers to computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis. Each step of this process will be monitored through the implementation of individual passwords and/or regular backups in order to maintain appropriate database access and to guarantee database integrity.

12.2.1 Data Management System

We will be using the REDcap software. The majority of collected data is directly entered into this eCRF and password protected.

REDCap is a secure web application for data collection, with audit trail.

12.3 External monitoring

Monitoring will be performed according to ICH Good Clinical Practice (GCP) by the Swiss Tropical and Public Health Institute under the direct supervision of the Sponsor. Following a Monitoring Plan and written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. They will perform this role by regular visits to the trial site, direct observation of a participant advancing through the trial pipeline and regularly reviewing reporting documentation.

12.4 Audits and inspections

The study documentation and the source data/documents are accessible to auditors/inspectors (also CEC and CA) and questions are answered during inspections. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

Once the follow up period of a patient is terminated, all data will be coded: volunteer data will be identified by a unique study number in the CRF and database. A separate independent confidential file containing identifiable information will be stored in a secured location in accordance with data protection requirements. Only the Sponsor representatives, Investigators, the clinical monitor, the CEC and regulatory authorities will have access to the records.

Photographs taken of local vaccination reactions, rashes or adverse events requiring such documentation (if required, with the volunteer’s written informed consent) will not include the volunteer’s face and will be identified by the date, trial code and participant’s unique coded identifier. Photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

If a study participant is volunteering to communicate with the media, he/she is free to do so on his/her own initiative and responsibility.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the Sponsor. All this information will be given without any names or confidential personal information.

12.6 Storage of biological material and related health data

Biological samples will be kept for 10 years in coded tubes in a secured medical-grade laboratory in CHUV (Department of immunology, CHUV, Lausanne), specific analyses may be outsourced to specialised collaborating laboratories.

All transfers will be subject to an MTA with conditions specifying the users respect the procedures and ethical standards of this protocol and legal jurisdiction.

According to ClinO art. 45, the Sponsor will keep all data relating to the clinical trial until the expiry date of the last batch of the tested IMP or for at least ten years from the end or the stopping the clinical trial.

13. PUBLICATION AND DISSEMINATION POLICY

The Investigators will be involved in writing and/or reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Apart from obvious flaws to the conduct of the study, which may preclude data publication, safety and efficacy data will be published under the supervision and authorization of PI and Sponsor.

Authorship follows the ICJME principles

14. FUNDING AND SUPPORT

The study will be entirely funded by the Sponsor according to the contract signed between Emergex Vaccines Holding Limited and Unisanté.

15. INSURANCE

The potential damages caused to participants will be supported by Newline, represented in Switzerland by Lloyd Switzerland (Seefeldstrasse 7, 8008 Zurich) underwriting contracted by the Sponsor of the study, in accordance with applicable law.

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16. APPENDICES

16.1 APPENDIX A: Solicited local and systemic AE

Adapted from FDA, available here: https://www.fda.gov/media/73679/download

16.1.1 Solicited Local AE

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
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

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

16.1.2 Solicited Systemic AE

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of nonnarcotic pain reliever or prevents daily activity
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity
Fever (°C) by axillary temperature	37.5 – 37.9	38.0 – 38.4	>38.5

16.2 APPENDIX B: Severity grading for abnormal laboratory measures

Adapted from CTCAE v5.0, available here: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf, available here: https://www.fda.gov/media/73679/download

LABORATORY TEST	*UNIT*	***REF RANGE***	GRADE 1	GRADE 2	GRADE 3	GRADE 4	GRADE 5
Hb ↓ (anaemia)	*g/l*	*♀117-157 ♂133-177*	<117 – 100	<100 – 80	< 80	Life threatening consequences, urgent intervention indicated	Death
WBC ↑ (leucocytosis)	*G/l*	*4.0-10*	10.8 – 15.0	15.1 – 20.0	20.0 – 25.0		
WBC ↓ (leukopenia)	*G/l*	*4.0-10*	< 3.5 – 2.5	<2.5 – 1.5	<1.5		
Neutrophils ↓ (neutropenia)	*G/l*	*1.8-7.5*	<1.8 – 1.5	<1.5 – 1.0	<1.0		
Lymphocytes ↓ (lymphopenia)	*G/l*	*1.5- 4.0*	<1.5 – 0.8	<0.8 – 0.5	<0.5		
Eosinophils ↑ (eosinophilia)	*G/l*	*0.05-0.5*	0.5 – 1.5	1.5 – 5.0	>5.0		
Platelets ↓ (thrombopenia)	*G/l*	*150-350*	<150 – 125	<125 – 75	<75 OR presence of clinical signs and symptoms of spontaneous bleeding		
Creatinine ↑	*umol/l*	*♀44-80 ♂62-106*	>ULN –1.5 x ULN	>1.5–2.5 ULN	>2.5 x ULN		
Urea ↑	*mmol/l*	*♀2.9-6.4 ♂2.9-7.7*	8.2 – 8.9	9.0 – 11.0	>11.0		
ALT, AST ↑	*U/L*	*9-50*	51 – 150	>150 – 250	>250		
Alkaline phosphate ↑	*U/L*	*36-120*	121 – 300	>300 – 600	>600		
GGT ↑	*U/l*	*6-42*	43 – 130	131 – 210	>210		
Total Bilirubin ↑	*umol/l*	*0-21*	22 – 31	32 – 63	>63		
C reactive protein↑	*mg/l*	*0-10*	10 – 30	30-80	>80		
Antinuclear antibodies	*Titer*	*Patient baseline*	1/1280 or >4 fold increase from baseline	-	-		

Note: the standard analysis panel for "full blood count" includes several measures that are taken and not listed here as part of the analysis of abnormalities (i.e. haematocrit, RBC, MCV, monocytes, basophils)

These additional measures will help indicate the clinical subtype of a given anomaly or provide clinical context, but are not specifically reported.

Urinalysis at screening will be assessed as per the table below:

URINALYSIS	
Protein*	1+
Blood**	1+ on two dipstick tests
Glucose	1+

**In the event of the dipstick testing positive for protein with ≥1+ protein urine should be sent for a protein creatinine ratio.*

*** In the event of urine dipstick testing positive for ≥1+ blood with, or without, protein in volunteers a repeat dipstick test will be carried out to confirm haematuria. In female volunteers, a menstrual history will be taken to elicit whether the subject is currently menstruating and if they are, urine dipstick will be repeated after 1 - 2 weeks. If blood and/or proteinuria persist in any volunteer, they will be excluded from the trial, and the appropriate follow-up arranged.*

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