A Dose Finding Study for the Assessment of Delayed-type Hypersensitivity Reactions to SARS-CoV-2 Peptide Antigens in Uninfected Healthy Subjects, COVID-19 Convalescent Subjects, and COVID-19 Vaccinated Subjects "COVID-19 DTH"

Sponsor:	Tonix Pharmaceuticals, Inc. [redacted]
Contract Research Organization:	Premier Research [redacted]
Sponsor Study Number:	TNX-CA-C201
US IND Number:	27509
Product Name:	TNX-2100
Development Phase:	Proof-of-Concept, Dose finding
Version (Date) of Final Protocol (Amendment 01):	Final, 07 December 2021

This clinical study will be conducted in accordance with the International Council for Harmonisation Tripartite Guideline for Good Clinical Practice (GCP) E6(R2), the protocol and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor

Protocol Title: A Dose Finding Study for the Assessment of Delayed-type Hypersensitivity Reactions to SARS-CoV-2 Peptide Antigens in Uninfected Healthy Subjects, COVID-19 Convalescent Subjects, and COVID-19 Vaccinated Subjects "COVID-19 DTH"

This clinical study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the guidelines on Good Clinical Practice (GCP) applicable to this clinical study.

Sponsor Signatory

[redacted]

Date

SIGNATURE PAGE

Declaration of the Principal Investigator

Protocol Title A Dose Finding Study for the Assessment of Delayed-type Hypersensitivity Reactions to SARS-CoV-2 Peptide Antigens in Uninfected Healthy Subjects, COVID-19 Convalescent Subjects, and COVID-19 Vaccinated Subjects "COVID-19 DTH"

I have read the TNX-CA-C201 protocol and agree to conduct the study as outlined. This clinical study protocol was subjected to critical review and has been released by the Sponsor. The information it contains is consistent with current risk and benefit evaluation of the investigational product, as well as with the moral, ethical and scientific principles governing clinical research as set out in the guidelines on GCP applicable to this clinical study.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Principal Investigator

[redacted]

Date

LIST OF STUDY STAFF

PROCEDURES IN CASE OF EMERGENCY

2. SYNOPSIS

Title of Study: A Dose Finding Study for the Assessment of Delayed-type Hypersensitivity Reactions to SARS-CoV-2 Peptide Antigens in Uninfected Healthy Subjects, COVID-19 Convalescent Subjects, and COVID-19 Vaccinated Subjects "COVID-19 DTH"
Study Number: TNX-CA-C201
Sponsor: Tonix Pharmaceuticals, Inc.
[redacted]
Study center(s): Up to three centers in the United States (US) will participate in this study.

Estimated Studied period: Estimated date first subject enrolled: Dec 2021 Estimated date last subjects completed: July 2022	**Phase of development:** Proof-of-Concept, Dose finding

Objectives:

Primary

- To evaluate the safety of intradermally-injected synthesized TNX-2100 SARS-CoV-2 peptide antigens.
- To assess the presence of delayed-type hypersensitivity (DTH) reactions in response to intradermal injection of synthesized TNX-2100 SARS-CoV-2 peptide antigens.

Secondary

- To estimate sensitivity and specificity of TNX-2100 as a marker of recovered infection relative to clinical history of infection.
- To identify the optimal synthesized SARS-CoV-2 peptide antigens and concentration for sensitive and specific DTH reaction.
- To identify the optimal timepoint for assessment of the DTH reaction.
- To correlate the DTH reaction with the adaptive T-cell immune response to SARS-CoV-2.

Methodology: This is a Proof-of Concept (POC), dose finding, multi-cohort study designed to evaluate the safety of intradermally-injected synthesized TNX-2100 SARS-CoV-2 peptide antigens and assess the presence of DTH reactions in response to intradermal injection of TNX-2100 synthesized SARS-CoV-2 peptide antigens. This study will be conducted in a total of approximately 90 subjects (30 subjects per cohort) who are either uninfected/unexposed healthy individuals (Cohort 1), who are confirmed to have recovered from SARS-CoV-2 infection, independent of vaccination status (Cohort 2) or who have received a complete SARS-CoV-2 vaccine course with no known history of natural infection (Cohort 3). The study will be conducted at 1-3 investigational sites in the US.

The study Schedule of Activities will consist of a Baseline/Skin Test Administration at Visit 1 (Day 1) at which time baseline assessments are to be completed, the skin test is performed, and appropriate test

samples are collected. Follow-up visits to monitor safety and evaluate the presence or absence of DTH reactions will be conducted per the following schedule: Visit 2 (Day 2); Visit 3 (Day 3); Visit 4 (Day 4); Visit 5 (Day 5). Safety monitoring will continue at Visit 6 (Day 30) and Visit 7 (Day 180) by telephone calls.

Eligible subjects who provide written informed consent and who satisfy eligibility criteria will undergo a nasopharyngeal swab for a polymerase chain reaction (PCR) (reverse transcriptase [RT]-PCR or other) test to detect active SARS-CoV-2 infection. A laboratory PCR test or a sponsor approved rapid PCR test (such as the Accula™ RT-PCR test) may be used. If a laboratory PCR test is used, subjects will complete a Screening Visit between Day -2 and Day -1 to allow processing of the sample prior to the Baseline/Skin Test Administration Visit 1 (Day 1). The rapid PCR test can be performed at Visit 1 (Day 1) and is preferred to facilitate enrollment by reducing the number of required site visits. If the test result is negative, the subject will proceed with the required blood draws followed by intradermal injection of the investigational products (IPs) and controls.

The estimated study duration will be approximately six months. In addition, unscheduled visits may occur based on Investigator assessment and /or Sponsor Medical Monitor request, or as clinically indicated. Photography of the injection site (volar forearms) after IP administration at Visit 1 and at Visit 3, 4, and 5, or unscheduled in person visits, will be used for safety documentation of local skin reaction, such as redness and swelling, or unexpected findings such as rash, blistering and necrosis, which require assessment by the designated Investigator.

A total of eight vials are provided, per subject. Three of the eight vials will be the ‘undiluted’ TNX-2110, TNX-2120 and TNX-2130 sterile solution (2.5 µg/mL) for injection. One vial will be the positive control CANDIN®. The remaining four vials are Diluent containing phosphate buffer pH 7.0 with 0.31% polysorbate 20 and 4.6% mannitol. Of these Diluent vials, three of them will be labeled as ‘1:10 Dilution TNX-2110’, ‘1:10 Dilution TNX-2120’, and ‘1:10 Dilution TNX-2130’ to represent the three “diluted” IPs. The remaining Diluent vial will be used as the negative control.

Intradermal injections will be administered in two stages in the order shown in the table below Table 2. Three IPs (TNX-2110, TNX- 2120, TNX-2130) will be administered by intradermal injection (0.1 mL) in two concentration strengths (Stage 1: “1:10 dilution” and Stage 2: “undiluted”). Subjects will also receive one intradermal injection (0.1 mL) of a positive control (CANDIN®), and one intradermal injection (0.1 mL) of a negative control “diluent”.

In total, each subject will receive eight intradermal injections (four per forearm), spaced 2 inches apart at pre-determined sites on the volar aspect of the forearms.

Table 2: Administration of In Vivo Investigational Products and Intradermal Controls

	To be administered intradermally in the following numerical order	
	LEFT arm	***RIGHT arm***
Stage 1	① Negative control (0.1 mL diluent) IP at 'Diluted' dose[a] ② TNX-2110 ③ TNX-2120 ④ TNX-2130	⑤ Positive control (0.1 mL CANDIN®)
Safety Monitoring Period		
Stage 2		IP at 'Undiluted' dose[b] ⑥ TNX-2110 ⑦ TNX-2120 ⑧ TNX-2130
Safety Monitoring Period		

[a] 'Diluted' dose = 0.1 mL (0.025 µg peptide per 100 µL; concentration strength "1:10 dilution")
[b] 'Undiluted' dose = 0.1 mL (0.25 µg peptide per 100 µL; concentration strength "undiluted")
IP = Investigational Product

Stage 1:

Subjects will receive the diluent (negative control), followed by the "1:10 dilution" strength of TNX-2110, TNX-2120, TNX-2130 in their left forearm, and CANDIN® (positive control) in their right forearm in the order and at the specific locations shown in Table 2 and described in the Pharmacy Manual (Appendix 5).

After Stage 1 administration, subjects will be monitored for local site reactions and systemic adverse reactions for 60 minutes. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. At 30 minutes post-administration, vital signs (systolic and diastolic blood pressure, heart rate, temperature) will be measured. If no systemic adverse reactions or unusual local site reactions per the Investigator's judgement are observed after 60 minutes, the subjects will proceed to Stage 2 administration.

Stage 2:

Subjects will receive the "undiluted" dose strength of TNX-2110, TNX-2120, TNX-2130 in the right forearm in the order and at the specific location indicated in Table 2.

After Stage 2 administrations, subjects will be monitored over the course of 30 minutes for unusual local site reactions and systemic adverse reactions. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. At 30 minutes post-administration, vital signs will be assessed. If no evidence of systemic adverse reactions or unusual local site reactions, per the Investigator's judgement, have occurred, subjects will be free to leave the clinic and will be contacted 24

hours later for a brief telephone or in-person safety follow-up visit (Visit 2, Day 2). Subjects will be instructed to keep the injection sites clean, uncovered, and to not scratch or rub the area.

Clinically significant symptoms and other adverse events (AEs) will be assessed by the Investigator as defined in the Food and Drug Administration (FDA) guidance for industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening). Adverse events of systemic allergic reaction will be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (see Appendix 1).

Local injection site skin reactions will be captured by subject self-reported 7-point Likert scales indicating the severity of erythema (redness), pain, pruritis (itching), and swelling.

Medications and equipment (crash cart and adrenaline auto-injectors [EpiPen, Jext or Emerade]) to manage possible anaphylactic reactions will be available for immediate use. All investigative site staff will be trained in identifying anaphylaxis and a medically licensed professional trained in the clinical management of anaphylaxis will be present at the skin test administration visit (Visit 1, Day 1) for the duration of Stages 1 and 2.

Subjects will be asked to return to the clinic for Investigator's in person assessment of the skin test result at various time points starting with 48 hours post skin test administration (Visit 3, Day 3) followed by two subsequent visits at 72 hours (Visit 4, Day 4) and at 96 hours (Visit 5, Day 5) post skin test administration. In addition, subjects will have two telephone safety follow-up visits (Visit 6, Day 30 and Visit 7, Day 180).

The duration of the study will be approximately six months, for each subject.

Blood samples will be taken for the detection and quantification of antibodies to SARS-CoV-2. Flow cytometric analysis of SARS-CoV-2-specific T-cell responses will be measured, along with in vitro positive controls. In addition, characterization of Th17 response will be evaluated using an enzyme-linked immune assay. A blood sample will also be taken for analysis using the T-Detect COVID test to aid the identification of individuals with an adaptive T-cell immune response to SARS-CoV-2, thus indicating recent or prior infection with SARS-CoV-2.

Investigational product, dosage and mode of administration: Three IPs (TNX-2110, TNX-2120 and TNX-2130) each containing a number of synthetic peptides as well as compendial excipients. Subjects will receive 0.1 mL intradermal doses of 0.025 and 0.25 µg per 100 µL (two potential strengths at a relation of "1:10 dilution" and "undiluted" product) per peptide. The dosing plan has been derived from the tuberculin DTH test with a strength of 0.1 µg antigen per 100 µL.

Positive and Negative controls, dosage and mode of administration: The positive control will be commercially available Candida albicans antigens (CANDIN®) and will be administered intradermally (0.1 mL). The negative control (diluent) will consist of phosphate buffer with 0.31% polysorbate 20 and 4.6% mannitol and will be administered intradermally (0.1 mL).

Further in vitro positive controls, PepMIX™ Candida (MP65) - JPT Peptide Technologies containing candida albicans peptides and a Tetanus Toxin Peptide Pool, will also be analyzed to confirm that subjects have intact T-cell immunity and are not immunodeficient.

Study Population: Male and female adults, aged 18 to 65 years of age, inclusive. Subjects will be uninfected/unexposed healthy individuals, SARS-CoV-2 convalescent subjects, or SARS-CoV-2 vaccinated subjects.

Number of subjects (planned): Approximately 90 adult subjects will be enrolled in parallel into three cohorts according to the inclusion/exclusion criteria:

- Cohort 1: 30 healthy uninfected/unexposed subjects.
- Cohort 2: 30 subjects who have recovered from SARS-CoV-2 infection at least two months prior to enrolment into the study independent of vaccination status.
- Cohort 3: 30 subjects who have received a complete SARS-CoV-2 vaccine course at least four weeks prior to enrolment into the study with no known history of natural infection.

A complete SARS-CoV-2 vaccine course is 1 dose of the Johnson & Johnson/Janssen COVID-19 vaccine OR 2 doses of either the Moderna or Pfizer-BioNTech COVID-19 vaccine. To be eligible for this study, subjects must have received their final vaccination dose at least 28 days (4 weeks) prior to enrolment. Subjects who have received a COVID-19 vaccine booster dose are also eligible for enrolment if at least 28 days (4 weeks) have elapsed since administration of booster.

Inclusion Criteria:

Subjects who meet the following criteria will be considered eligible to participate in the clinical study:

1. Male or female subjects aged 18 - 65 years of age, inclusive, in good general health as determined by medical evaluation (medical history, physical examination, vital signs, 12-lead electrocardiogram [ECG] and clinical laboratory evaluations).
2. Females who are not of childbearing potential (defined as at least 12 months natural spontaneous amenorrhea, or at least 6 weeks following surgical menopause) or females of childbearing potential who agree to comply with the contraceptive requirements of the protocol.
3. Subject is willing and able to provide written informed consent prior to performing study procedures and be able to read and understand the study protocol.
4. Subject understands and agrees to comply with all planned study procedures.
5. Subject's right and left forearms must be free of large tattoos or skin abnormalities that would interfere with skin test administration and assessment in the opinion of the Investigator.
6. For healthy uninfected subjects (Cohort 1): Must have no history of signs or symptoms consistent with SARS-CoV-2 infection at any time within six months of enrollment AND in the Investigator's opinion, have no known intimate exposure or close contact to persons confirmed positive for SARS-CoV-2 infection.
7. For recovered subjects (Cohort 2): Must be > 2 months removed from confirmed diagnosis of SARS-CoV-2 infection independent of vaccination status.
8. For subjects who have received a SARS-CoV-2 vaccine (Cohort 3): must have received their final vaccination or booster dose at least 28 days (four weeks) prior to enrolment into the study with no known history of natural infection.

9. Subject receives a negative SARS-CoV-2 PCR test result at their screening or baseline visit.

Exclusion Criteria:

Subjects who meet one or more of the following criteria will not be considered eligible to participate in the clinical study:

1. Female subjects who are pregnant or breastfeeding or planning to breastfeed at any time through 90 days after the Screening Visit.
2. Diagnosed with clinically significant and currently relevant cardiac disease:
    - Significant arrhythmia; heart block; heart failure; symptomatic coronary artery disease), recent myocardial infarction [within the past two years]) or QTcF >450 msec (male) or >470 msec (female).
3. Presence of erythema nodosum, eczema, psoriasis, or cellulitis.
4. Treatment with systemic corticosteroids, immunosuppressive drugs or any anti-viral treatment within two weeks prior to Screening.
5. History of immunosuppressive disease.
6. Prior adverse reaction to phlebotomy.
7. Prior adverse reaction to CANDIN® or similar material.
8. History of hypersensitivity to the study materials used for phlebotomy (i.e., latex, rubbing alcohol, cotton swabs, band-aids).
9. Documented allergic reaction to any of the excipients contained in the IP (e.g., polysorbate).
10. Subjects with dermatographism.
11. Subjects will be excluded if they have clinically significant underlying conditions associated with high risk for severe COVID-19 infections as identified by the Centers for Disease Control and Prevention (CDC) (Appendix 2). These conditions include, but are not limited to: chronic obstructive pulmonary disease, diabetes mellitus (Type 1 and 2), obesity, hypertension, heart disease, and cerebrovascular disease.
    - Investigators should contact the Medical Monitor with questions regarding clinical significance of underlying conditions.
12. The subject has any clinically significant, uncontrolled, or unstable medical or surgical condition that could affect his or her ability to participate in the study or potentially compromise his or her well-being during the study.
13. Unable to understand the protocol requirements, instructions and study related restrictions, the nature, scope and possible consequences of the clinical study.
14. Healthy uninfected subjects who have any history of SARS-CoV-2 infection by clinical history or laboratory diagnosis (**Cohort 1 only**).

Criteria for Evaluation:

Safety:

Safety of intradermally administered TNX-2100 SARS-CoV-2 peptides, as measured by:

- Incidence of AEs.
    - Clinically significant symptoms and other AEs will be assessed by the Investigator as defined in the FDA Guidance for Industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening).
    - Adverse events of systemic allergic reaction will be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (see Appendix 1).
    - Local injection site skin reactions will be captured by subject self-reported 7-point Likert scales indicating severity of: erythema, pain, pruritis and swelling.
- Protocol-defined adverse events of special interest (AESI):
    - Anaphylactic reaction;
    - Enhanced disease from repeated exposure to SARS-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

Efficacy:

The following study variables will be evaluated to assess the efficacy of intradermally administered TNX-2100 SARS-CoV-2 peptides:

Primary efficacy endpoint:

- Maximal area of induration ≥5 mm at injection sites on the volar forearms at 48 hours, 72 hours and 96 hours post skin test administration.

Secondary efficacy endpoints:

- Correlation of DTH reaction to clinical history of prior SARS-CoV-2 infection.
- Correlation of DTH reaction to adaptive T-cell immune response to SARS-CoV-2 assessed by the T-Detect COVID Test.
- Correlation of DTH reaction with in vitro assays of immune response including SARS-CoV-2 antibody levels
- Characterization of Th1 and Th2 responses by measuring cytokine production and cell surface T-cell phenotype markers by intracellular cytokine staining.

Exploratory efficacy endpoint:

- Characterization of Th17 response by enzyme linked immune assay.

Study Halting and Stopping Rules:

Dosing may be halted temporarily to investigate before the entire study is terminated. If any of the below events occur, enrolment and dosing should be paused study-wide pending review by the Sponsor, Investigator, and/or Medical Monitor. Subjects enrolled in the study must continue to be followed for the duration of the study.

- One or more subjects with a serious adverse event (SAE) considered definitely, probably, or possibly related to the IPs.
- Three or more subjects with the same or similar grade 3 or higher AEs of the same type considered definitely, probably, or possibly related to IPs.
- One AESI, considered at least possibly related to the IPs.

Duration of Study

The duration of study involvement will be approximately six months.

Statistical methods:

Analysis Populations

The following analysis populations will be used during the study:

- Safety Population: includes any subjects in Cohort 1, 2, or 3 who receive at least 1 intradermal injection of the IPs.
- Intent-to-Treat (ITT) Population: includes any subjects in Cohort 1, 2, or 3 who provide written informed consent, satisfy all inclusion/exclusion criteria, and have a negative test result from PCR detection of SARS-CoV-2 viral RNA.
- Modified Intent-to-Treat (mITT) Population: includes subjects from ITT who complete stages 1 and 2 of administration and have induration responses for at least one post skin test administration visit at hour 48, 72, or 96.

All safety analyses will be conducted using the Safety Population. All AEs will be listed. The number and percent of subjects experiencing an event will be tabulated for each System Organ Class and Preferred Term. The AEs will also be tabulated according to severity and causality. Serious AEs (SAE) will be listed separately.

Efficacy analyses will be conducted using the mITT Population. The frequency and percentage of subjects with a maximal area of induration of ≥5 mm will be summarized within each cohort and by overall subjects.

Cytokine and cell surface T-cell phenotype markers such as COVID-19 antibody titers will be summarized descriptively within each cohort and by overall subjects. Enzyme linked immune assay parameters will be summarized descriptively within each cohort and by overall subjects.

Individual data will be listed. Data will be summarized using suitable descriptive statistics. Depending on the structure of the data either sample statistics or frequency tables will be generated.

Additional analyses details will be provided in the final Statistical Analysis Plan (SAP).

Interim Analysis

The skin test DTH result assessments are obtained at Visits 3, 4 and 5 (48, 72 and 96 hours post administration of skin test). An interim analysis will be conducted after all subjects have completed Visit 5 (or early terminated prior to Visit 5), and a database lock of the efficacy and safety data up to Day 5 will occur. The purpose of the interim analysis will be to assess the presence of DTH reactions in response to intradermal injection of synthesized TNX-2100 SARS-CoV-2 peptide antigens, and to assess safety up to 96 hours post-administration. A full database lock will occur after all subjects have completed Visit 7 (or early terminated prior to Visit 7).

Sample Size

Formal sample size calculations were not performed. The number of subjects were chosen based on feasibility and are considered sufficient to meet the study objectives.

Table 3: Schedule of Activities

Activity	Screening Visit	Baseline/Skin Test Administration Visit	Visits[a]					
Visit	**Screening**	**Visit 1 (Skin Test Administration)**	**Visit 2 (Subject has option of in person or telephone safety monitoring visit)[b]**	**Visit 3 (In-person for assessment of skin test result)[c]**	**Visit 4 (In-person assessment of skin test result and safety FU)[d]**	**Visit 5 (In-person assessment of skin test result and safety FU)[e]**	**Visit 6 ☎ (Telephone safety FU)[f]**	**Visit 7 ☎ (Telephone safety FU)[g]**
Day	**Days – 2 to -1**	**Day 1**	**Day 2 24 h post skin test**	**Day 3 48 h post skin test**	**Day 4 72 h post skin test**	**Day 5 96 h post skin test**	**Day 30 30 days post skin test**	**Day 180 180 days post skin test**
Visit Window	**NA**	**NA**	**NA**	**±4 h**	**±4 h**	**±4 h**	**± 5 days**	**± 5 days**
Informed consent	X	X						
Inclusion/exclusion		X						
Demographic data		X						
Medical history		X						
Prior and concomitant medications		X					X	X
Vital signs[h]		X						
12-lead electrocardiogram[i]		X						
Physical examination		X						
Nasopharyngeal swab for detection of SARS-CoV-2[j]	X	X (ONLY subjects who did not complete at Screening Visit)						
Clinical symptoms/signs preceding SARS-CoV-2 diagnosis [k]		X						
Time from onset of clinical signs of SARS-CoV-2 [k]		X						
Maximum SARS-CoV-2 severity experienced or contact (WHO Ordinal Scale for Severity) [k]		X						

Time from onset of exposure to person with SARS-CoV-2 [k]		X						
Therapies received for SARS-CoV-2 [k]		X						
On-site urine pregnancy test for female subjects of child-bearing potential		X						
Phlebotomy: Clinical laboratory tests[l]		X						
Phlebotomy: Whole blood for the T-Detect COVID Test		X						
Phlebotomy: Serum to measure SARs-CoV-2 antibody levels (ECLIA)		X						
Phlebotomy: Whole blood for characterization of the Th17 response		X						
Phlebotomy: Whole blood for ICS/Flow Cytometry		X						
IP Administration		X						
Adverse event monitoring; note that photography may be used as documentation of unusual local skin reactions		X	X	X	X	X	X	X
Assessment and measurement of Skin Test DTH result is to be conducted in person[m]				X	X	X		
Photography of injection sites on forearm[n]		X	X[o]	X	X	X	X[o]	X[o]
Long-term safety follow-up[p]							X	X

AE = adverse event; COVID-19 = coronavirus disease 2019; DTH = delayed-type hypersensitivity; EU: Emergency Use; FU = follow-up; h = hour; ICS= Intracellular Cytokine Staining; IP=investigational products; NA = Not Applicable; PCR=polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

[a] Unscheduled visits to assess AEs and the DTH reaction may occur between Visit 1 and Visit 7, based on investigator assessment and /or Sponsor medical monitor request, or as clinically indicated. Vital signs will be repeated at unscheduled visits. Clinical laboratory tests or other assessments may be repeated at the discretion of the investigator.
[b] Visit 2 will occur approximately 24 hours after administration of the intradermal injection of peptides. Subjects will have the option of an in person visit or a phone visit.

[c] Visit 3 will occur in person approximately 48 hours after administration of the intradermal injection of peptides.
[d] Visit 4 will occur in person approximately 72 hours after administration of the intradermal injection of peptides.
[e] Visit 5 will occur in person approximately 96 hours after administration of the intradermal injection of peptides.
[f] Visit 6 will occur by telephone approximately 30 days after administration of the intradermal injection of peptides.
[g] Visit 7 will occur by telephone approximately 180 days after administration of the intradermal injection of peptides.
[h] Vital signs (systolic and diastolic blood pressure, pulse, body temperature, and respiratory rate) will be measured at the beginning of Visit 1 for a Baseline reading. Vital signs will also be measured during Stage 1 and Stage 2 of skin test administration. In Stage 1, vital signs will be measured 30 minutes following administration of the 3 x 0.025 µg peptides (dose strength "1:10 dilution" of IP), negative and positive control. If no systemic adverse reactions or unusual local site reactions per the Investigator's judgement are observed after 60 minutes, the subjects will proceed to Stage 2 for administration of the of 3 x 0.25 µg TNX-2100 peptides per 100 µL (undiluted product). After Stage 2 administrations, subjects will be monitored over the course of 30 minutes for unusual local site reactions and systemic adverse reactions. At 30 minutes post-administration, vital signs will be assessed. If no evidence of systemic adverse reactions or unusual local site reactions per the Investigator's judgement have occurred, subjects will be free to leave the clinic.
[i] A 12-lead electrocardiogram will be obtained at Visit 1. All electrocardiograms will be conducted and interpreted locally.
[j] A nasopharyngeal swab will be collected at a Screening Visit between Day -2 to -1 if laboratory PCR test used, or Visit 1 (Day 1) if rapid PCR test used for detection of active SARS-CoV-2 infection.
[k] Initially applicable to Cohort 2 (recovered subjects) only. Assessments will be required for any new COVID-19 infection.
[l] Clinical laboratory tests (hematology and serum chemistry) will be performed at Visit 1. Clinical laboratory tests may be repeated at the discretion of the investigator.
[m] Details regarding the assessment and measurement of the TNX-2100 Skin Test are provided in the Pharmacy Manual (Appendix 5).
[n] Photography of the injection site (volar forearms) after IP administration at Visit 1 and at Visit 3, 4, 5 and any unscheduled in person visits, will be used for safety documentation of local skin reaction, such as redness and swelling, or unexpected findings such as rash, blistering and necrosis, which require assessment by the designated Investigator. At Visit 1, the injection sites should be photographed after Stage 1, and again after Stage 2 (at the start of each safety monitoring period). Subject should lay arms flat, palms facing up. Site should photograph from above and in a well-lit area. Site should take photos uniformly for all subjects so that both forearms from inner elbow to wrist are visible.
[o] If subjects opt for an in-person visit at Visit 2 or Unscheduled Visit, photography of the injection sites (volar forearms) will be used for safety documentation of local site and any unexpected findings.
[p] Long term safety follow-up contact at Visit 6 and Visit 7 will consist of a brief medical history review and assessed for new or recent COVID-19 infection since the last visit. Unvaccinated subjects will also be asked to confirm any update to vaccination status. Subjects will be asked to confirm whether there is a complete resolution of DTH skin reaction to skin test peptides.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Term	Definition
AE	Adverse event
AESI	Adverse events of special interest
BMI	Body mass index
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CANDIN®	Candida albicans skin test antigen for cellular hypersensitivity
CBC	Complete blood count
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
CRF	Case report form
CRU	Clinical research unit
CRO	Contract research organization
DNA	Deoxyribonucleic acid
DTH	Delayed-type hypersensitivity
ECG	Electrocardiogram
ECLIA	Electrochemiluminescence Immunoassay
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GCP	Good clinical practices
GLP	Good laboratory practices
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leucocyte antigen
HPV	Human papillomavirus
HSV	Herpes simplex virus
ICF	Informed consent
ICH	International Council for Harmonisation

ICS	Intracellular cytokine staining
IL	Interleukin
IP	Investigational product
IRB	Institutional review board
ITT	
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
mITT	
MMR	Measles, mumps, rubella
PCR	Polymerase chain reaction
PBMC	Peripheral blood mononuclear cells
POC	Proof-of-Concept
PPD	Purified protein derivative
QTcF	QTc derived from Fridericia's Formula
RBC	Red cell count
RBD	Receptor binding domain
RDW	Red cell distribution width
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TB	Tuberculosis
Th	T helper cell
TT	Tetanus toxin
US	United States
WBC	White blood cell count
WHO	World Health Organization

5. INTRODUCTION

5.1. Overview of SARS-CoV-2

In late 2019, a new disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China that is now known as coronavirus disease 2019 (COVID-19). SARS-CoV-2 has emerged as an easily transmissible disease, affecting millions of people worldwide (Chan et al., 2020a; Chen et al., 2020; Li et al., 2020; Wang et al., 2020; Zhu et al., 2020).

The most common coronaviruses in clinical practice are 229E, OC43, NL63, and HKU1, which typically cause common cold symptoms in immunocompetent individuals. SARS-CoV-2, a member of the betacoronavirus genus, is closely related to SARS-CoV and several bat coronaviruses (Lu et al., 2020; Tan et al., 2020; Zhu et al., 2020).

The spectrum of SARS-CoV-2 ranges from asymptomatic forms to severe viral pneumonia with respiratory failure, multiorgan and systemic dysfunctions, and death (Guan et al., 2020, Huang et al., 2020). The majority (80%) of cases are mild and self-limiting; severe disease requiring hospitalization occurs in the remaining 20% mainly due to fulminant pneumonia and respiratory failure (Wu et al., 2020).

5.2. Background of TNX-2100

Knowledge of diagnostic tests for SARS-CoV-2 is still evolving, and a clear understanding of the nature of the tests and interpretation of their findings is important. To date, the most commonly used and reliable test for diagnosis of SARS-CoV-2 has been the reverse transcription polymerase chain reaction (RT-PCR) test performed using nasopharyngeal swabs or other upper respiratory tract specimens, including throat swab or, more recently, saliva. A variety of RNA gene targets are used by different manufacturers, with most tests targeting 1 or more of the envelope, nucleocapsid, spike, RNA-dependent RNA polymerase, and ORF1 genes. False negative results and lengthy wait times for molecular RT-PCR tests question their usefulness in determining active infection.

The only currently available methods to detect T cell immunity to SARS-CoV-2 require expensive, multi-step sample preparation and in vitro T cell stimulation in highly specialized laboratories using methods that have not been amenable to standardization. The limitations of current testing methods underscore the unmet need for a rapid, sensitive, and specific test that may indicate present or past infection with SARS-CoV-2 and potentially predict protective immunity.

Discovered in 1882 by Robert Koch, the delayed hypersensitivity skin test has been used for more than a century as a clinical test for cell-mediated immune reactions (Black, 1999). In the 1940s, Landsteiner and Chase proved that the reaction was mediated by the cellular and not the humoral arm of the immune system (Landsteiner and Chase, 1940). When small quantities of antigen are injected dermally, a hallmark response is elicited which includes induration, swelling and monocytic infiltration into the site of the lesion within 24 to 72 hours. This reaction has been shown to be absolutely dependent on the presence of memory T cells. Both the cluster of differentiation (CD)4+ and CD8+ fractions of cells have been shown to modulate a response.

The delayed type hypersensitivity (DTH) skin tests have been commonly used to detect cell-mediated immune responses to tuberculosis, fungal pathogens, and mumps virus. A skin test's potential to be predictive of the quality and durability of immune responses, or infection/re-infection risk, or aid in diagnosis of current or previous infection could provide a method of testing for cellular immune response comparable to the rapid, reliable and scalable antibody tests that currently exist. Because both antibody-mediated and cellular-mediated immune responses are important to protection against the virus, having a fast, reliable and scalable test for cellular immunity is critical.

Currently there is no standardized laboratory test available to measure T cell immune responses to CoV-2. T cell immunity to CoV-2 persists longer than antibody immunity, is sometimes present in the absence of a measurable antibody response and is believed to provide an important element of protection against serious illness after infection with SARS-CoV-2.

TNX-2100 skin test is designed to measure T cell immunity to SARS-CoV-2 and uses peptide antigens presented in sterile solution administered intradermally. It is based on DTH reactions elicited after approximately 48 hours in individuals with preexisting T cell immunity to peptides in that mixture. The skin test comprises three different mixtures of synthetic peptides, which are designed to represent different protein components of the SARS-CoV-2 virus. Individuals who have been infected by or exposed to CoV-2 would be expected to respond to one or more of the three mixtures.

The TNX-2100 skin test will complement the limited information that can be obtained from serological tests to provide clinicians, patients, employers and public health officials with information of potential diagnostic, safety and predictive significance in a timely and cost-effective manner. It has utility as a potential biomarker for cellular immunity and protective immunity, a method to stratify participants in COVID-19 vaccine trials, an endpoint in COVID-19 vaccine trials, a biomarker of durability of vaccines, and as a method to identify vaccine failure.

5.3. Scientific Rationale

Studies of immune responses to the SARS-CoV-2 virus involve antibody-mediated and cellular components. However, there is considerable individual variation in the pattern of these responses (Wolfel et al., 2020; Ni et al., 2020, Wen et al., 2020). Both appear to be necessary for the development of protective immunity, but their relative contributions are subjects of intense investigation. Effective vaccines that confer long-term immunity are likely to require both components of the immune response.

At present, assays for the detection and quantification of SARS-CoV-2-specific antibodies are in wide clinical use and have been scaled up to meet the needs of public health efforts on an international level. However, no comparable methodology exists to measure cell-mediated immunity. Cellular responses are currently measured in highly specialized laboratories using in vitro techniques to detect cytokine production following antigenic challenge. Such approaches measure surrogate markers of T cell reactivity that may not reflect functional in vivo immunity. In addition, these assays are expensive, time-consuming, and impossible to scale up for public health application.

Tonix Pharmaceuticals Inc. (hereafter referred to as the Sponsor) proposes to develop a scalable skin test (TNX-2100) based on the DTH reaction to demonstrate the presence of functional cell mediated immune responses to SARS-CoV-2.

The DTH skin test has long been the gold standard in the detection of cell-mediated immune responses. The general mechanism of the DTH response is based on the interaction of antigen with CD4+ and CD8+ lymphocytes followed by the secretion of interleukins and other lymphokines from macrophage cells. The release of effector molecules causes endothelial cells lining the blood vessels to become permeable and allows fibrinogen to escape into the surrounding tissue where it is converted to fibrin. The deposition of fibrin and the accumulation of T-cells and monocytes within the extracellular spaces cause the tissue to swell and become indurated. This process is usually detectable in 18 hours and peaks at 48 hours (Zweiman, 1998).

Recently, a study of DTH skin test in 51 subjects with history of SARS-Cov-2 infection was conducted (Barrios et al., 2021a). Using intradermal injections of recombinant SARS-Cov-2 Spike protein (and a Candida Albicans control), subjects were followed over 48 hours for development of skin induration at the injection site. Forty-three of the subjects had positive reactions at 48 hours. The 14 control subjects with no history of SARS-Cov-2 showed no reactivity to the Spike protein. Analysis of anti-receptor binding domain (RBD) IgG in the exposed subjects showed an 84.3% concordance with skin DTH results. A second study of DTH response to SARS-Cov-2 Spike protein was conducted pre- and post-vaccination of 28 subjects with 12 BNT162b2 mRNA (Pfizer)

vaccine (Barrios et al., 2021b). Eight of eleven subjects tested after a single dose of vaccine showed a positive DTH skin response. Twenty-eight of 28 subjects tested after two doses showed positive DTH skin reactions 14 days after the second vaccine dose. All subjects developed anti-RBD IgG antibodies.

The development of a DTH test for SARS-CoV-2 would provide clinicians with a straightforward way of evaluating the presence of functional cell-mediated immunity in patients with SARS-CoV-2. This information could assist in the diagnosis and clinical management of COVID-19 and help to evaluate the quality and durability of immune responses in recovering or convalescent SARS-CoV-2 patients.

The TNX-2100 skin test will be based on DTH reactions elicited using three sets of peptide antigens presented in sterile solution (TNX-2110, TNX-2120 and TNX-2130), which are designed to represent different protein components of the SARS-CoV-2 virus.

The TNX-2100 skin test is designed to be administered intradermally in the same way as skin tests for tuberculosis, or TB, sold as Tubersol® or Aplisol® or generically as the Mantoux tuberculin purified protein derivative (PPD) test. Each of these three synthetic peptides is expected to be administered as part of the same procedure, at separate locations on the forearm, and each is expected to elicit a DTH response after approximately 48 hours in individuals with pre-existing T cell immunity to peptides in that mixture. Individuals who have been infected by or exposed to SARS-CoV-2 would be expected to respond to one or more of these peptide mixtures.

The TNX-2100 skin test can easily be deployed in vaccine trials and large-scale public health efforts because it is easily utilized in large number of people and there are no requirements for complex laboratories, cold chain logistics etc. It is expected to provide clinicians, patients, employers and public health officials with information of potential diagnostic, safety and predictive significance in a timely and cost-effective manner, including the durability of immune responses.

The mixture of synthetic peptides that demonstrates optimal reactivity at the optimized dilution will be carried forward into a larger confirmatory study consisting of infected and uninfected subjects with the aim of implementing the optimized skin test within a late-phase vaccine trial for SARS-CoV-2.

5.4. Risk-benefit Assessment

The protocol has been designed to minimize the risk to research participants. Subjects will be monitored to detect adverse events (AEs) during the study and followed appropriately to ensure resolution of AEs.

Peptide-based products do not need in vitro culture, making them biologically safe, and their selectivity allows accurate activation of immune responses.

The skin test by intradermal injection is quick and virtually painless. The amount of allergen introduced into the skin is so small that a serious reaction is very unlikely. However, immediate hypersensitivity, to include severe systemic reactions, may occur following administration of skin test antigens. Medications and equipment (crash cart and adrenaline auto-injectors e.g. EpiPen, Jext or Emerade) to manage possible anaphylactic reactions will be available for immediate use. All investigative site staff will be trained in identifying anaphylaxis and a medically licensed professional trained in the clinical management of anaphylaxis will be present at the skin test administration visit (Visit 1, Day 1) for the duration of Stages 1 and 2. Subjects will be observed for a minimum of 30 minutes following administration to assess for adverse reactions.

Venipuncture to obtain blood samples of peripheral blood mononuclear cells (PBMCs) may be associated with discomfort. It is very rare for a blood test to result in serious complications; however, there is a very small possibility of complications arising (infection, excessive bleeding, mild bruising or hematoma and fainting/ dizziness).

The risk-benefit profile of the TNX-2100 skin test is considered to be acceptable for further evaluation and adequate monitoring of safety has been implemented into this protocol.

6. TRIAL OBJECTIVES

6.1. Primary Objectives

The main objectives of the study are to evaluate the safety of intradermally-injected synthesized TNX-2100 SARS-CoV-2 peptide antigens and to assess the presence of DTH reactions in response to intradermal injection of synthesized TNX-2100 SARS-CoV-2 peptide antigens.

6.2. Secondary Objectives

The secondary objectives are:

- To estimate sensitivity and specificity of TNX-2100 as a marker of recovered infection relative to clinical history of infection.
- To identify the optimal synthesized SARS-CoV-2 peptide antigens and concentration for sensitive and specific DTH reaction.
- To identify the optimal timepoint for assessment of the DTH reaction.
- To correlate the DTH reaction with the adaptive T-cell immune response to SARS-CoV-2.

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

This is a Proof-of-Concept (POC), dose finding, multi-cohort study designed to evaluate the safety of intradermally-injected synthesized TNX-2100 SARS-CoV-2 peptide antigens and assess the presence of DTH reactions in response to intradermal injection of synthesized TNX-2100 SARS-CoV-2 peptide antigens. This study is to be conducted in a total of approximately 90 subjects (30 subjects per cohort) who are either uninfected/unexposed healthy individuals (Cohort 1), who are confirmed to have recovered from SARS-CoV-2 infection independent of vaccination status (Cohort 2) or who have received a complete SARS-CoV-2 vaccine course with no known history of natural infection (Cohort 3). The study will be conducted at 1-3 investigational sites in the United States.

The study Schedule of Activities will consist of a Baseline/Skin Test Administration at Visit 1 (Day 1) at which time baseline assessments are to be completed, the skin test is performed, and appropriate test samples are collected. Follow-up visits to monitor safety and evaluate the presence or absence of DTH reactions will be conducted per the following schedule: Visit 2 (Day 2); Visit 3 (Day 3); Visit 4 (Day 4); Visit 5 (Day 5). Safety monitoring will continue at Visit 6 (Day 30) and Visit 7 (Day 180) by telephone calls.

Eligible subjects who provide written informed consent and who satisfy eligibility criteria will undergo a nasopharyngeal swab for a polymerase chain reaction (PCR) (reverse transcriptase [RT]-PCR or other) test to detect active SARS-CoV-2 infection. A laboratory PCR test or a sponsor approved rapid PCR test (such as the Accula™ RT-PCR test) may be used. If a laboratory PCR test is used, subjects will complete a Screening Visit between Day -2 and Day -1 to allow processing of the sample prior to the Baseline/Skin Test Administration Visit 1 (Day 1). The rapid PCR test can be performed at Visit 1 (Day 1) and is preferred to facilitate enrolment by reducing the number of required site visits. If the test result is negative, the subject will proceed with the required blood draws followed by intradermal injection of the investigational products (IPs) and controls.

A total of eight vials are provided, per subject. Three of the eight vials will be the ‘undiluted’ TNX-2110, TNX-2120 and TNX-2130 sterile solution (2.5 µg/mL) for injection. One vial will be the positive control CANDIN®. The remaining four vials are Diluent containing phosphate buffer pH 7.0 with 0.31% polysorbate 20 and 4.6% mannitol. Of these Diluent vials, three of them will be labeled as ‘1:10 Dilution TNX-2110’, ‘1:10 Dilution TNX-2120’, and ‘1:10 Dilution TNX-2130’ to represent the three “diluted” IPs. The remaining Diluent vial will be used as the negative control.

Intradermal injections will be administered in two stages (Stage 1: "1:10 dilution" and Stage 2: "undiluted") and each subject will receive a total of eight intradermal injections, spaced two inches apart at pre-determined sites on the volar aspect of both forearms. A detailed Pharmacy Manual outlining the IP administration and DTH assessment process will be provided to investigative site staff and is included in Appendix 5. Details of the skin test administrations are illustrated in Sections 12.1.10 and 12.1.10, respectively. Subjects will be contacted 24 hours later for a brief telephone or in-person safety follow-up visit (Visit 2, Day 2).

Each site will have medications and equipment (eg, crash cart and adrenaline auto-injectors [EpiPen, Jext or Emerade]) available for immediate use to manage possible anaphylactic reactions (rapidly developing, life-threatening problems involving the airway [pharyngeal or laryngeal edema] and/or breathing [bronchospasm with tachypnoea] and/or circulation [hypotension and/or tachycardia] and, in most cases, associated skin and mucosal changes). All investigative site staff will be trained in identifying anaphylaxis and medically licensed professional trained in the clinical management of anaphylaxis will be present at the skin test administration visit (Visit 1, Day 1) for the duration of Stages 1 and 2.

Local injection site skin reactions will be captured by a subject self-reported 7-point Likert scale indicating severity of: erythema (redness), pain, pruritis (itching), and swelling.

Clinically significant symptoms and other AEs will be assessed by the Investigator as defined in the Food and Drug Administration (FDA) guidance for industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening). Adverse events of systemic allergic reaction will also be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (Appendix 1).

Subjects will be asked to return to the site for an Investigator's in-person assessment of the skin test result at various time points starting with 48 hours post skin test administration (Visit 3, Day 3) followed by subsequent visits at 72 hours (Visit 4, Day 4) and at 96 hours (Visit 5, Day 5) post skin test administration. Photography of the injection site (volar forearms) after IP administration at Visit 1 and at Visit 3, 4, and 5, or unscheduled in person visit, will be used for safety documentation of local skin reaction, such as redness and swelling, or unexpected findings such as rash, blistering and necrosis, which require assessment by the designated Investigator.

In addition, subjects will have two telephone safety follow-up visits (Visit 6, Day 30 and Visit 7, Day 180) and unscheduled visits may occur based on Investigator assessment and /or Sponsor Medical Monitor request, or as clinically indicated.

The estimated study duration will be approximately six months.

7.2. Study Endpoints

7.2.1. Safety Endpoints

Safety of intradermally administered TNX-2100 SARS-CoV-2 peptides, as measured by:

- Incidence of AEs.
    - Clinically significant symptoms and other AEs will be assessed as defined in the FDA Guidance for Industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (FDA, 2007) using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening).
    - Adverse events of systemic allergic reaction will be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (Appendix 1).
    - Local skin reactions will be captured by a subject self-reported 7-point Likert scales indicating severity of: erythema, pain, pruritis and swelling.
- Protocol-defined adverse events of special interest (AESI):
    - Anaphylactic reaction.
    - Enhanced disease from repeated exposure to SARS-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

7.2.2. Efficacy Endpoints

The following study variables will be evaluated to assess the efficacy of intradermally administered TNX 2100 SARS-CoV-2 peptides:

7.2.2.1. Primary Efficacy Endpoint

- Maximal area of induration ≥ 5 mm at injection sites on the volar aspect of the forearms at 48 hours, 72 hours and 96 hours post skin test administration.

7.2.2.2. Secondary Efficacy Endpoints

- Correlation of DTH reaction to clinical history of prior SARS-CoV-2 infection.
- Correlation of DTH reaction to adaptive T-cell immune response to SARS-CoV-2 assessed by T-Detect COVID Test (Adaptive Biotechnologies).

- Correlation of DTH reaction with in vitro assays of immune response including SARS-CoV-2 antibody levels.
- Characterization of Th1 and Th2 responses by measuring cytokine production and cell surface T-cell phenotype markers by intracellular cytokine staining.

7.2.2.3. Exploratory Efficacy Endpoint

- Characterization of Th17 response by enzyme linked immune assay.

7.3. Number of Subjects and Treatment Assignment

A sufficient number of subjects will be enrolled to enable approximately 90 adult subjects to be enrolled in parallel into three cohorts according to the inclusion/exclusion criteria:

- **Cohort 1**: 30 healthy uninfected/unexposed subjects.
- **Cohort 2**: 30 subjects who have recovered from SARS-CoV-2 infection at least 2 months prior to enrolment into the study independent of vaccination status.
- **Cohort 3**: 30 subjects who have received a complete SARS-CoV-2 vaccine course at least four weeks prior to enrolment into the study with no known history of natural infection.

A complete SARS-CoV-2 vaccine course is 1 dose of the Johnson & Johnson/Janssen COVID-19 vaccine OR 2 doses of either the Moderna or Pfizer-BioNTech COVID-19 vaccine. To be eligible for this study, subjects must have received their final vaccination dose at least 28 days (4 weeks) prior to enrolment. Subjects who have received a COVID-19 vaccination booster dose are also eligible for enrolment if at least 28 days (4 weeks) have elapsed since administration of booster.

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Informed Consent

A potential subject may be screened for eligibility only after the nature of the study, its purpose, and any other information relevant to the subject's decision to participate have been explained to him or her and the subject has voluntarily confirmed his or her willingness to participate. Informed consent is documented by means of a written, signed, and dated informed consent form (ICF). Additional information is provided in Section 15.4.

8.2. Subject Inclusion Criteria

Subjects who meet the following criteria will be considered eligible to participate in the clinical study:

Inclusion Criteria:

1. Male or female subjects aged 18 - 65 years of age, inclusive, in good general health as determined by medical evaluation (medical history, physical examination, vital signs, 12-lead electrocardiogram [ECG] and clinical laboratory evaluations).

2. Females who are not of childbearing potential (defined as at least 12 months natural spontaneous amenorrhea, or at least six weeks following surgical menopause) or females of childbearing potential who agree to comply with the contraceptive requirements of the protocol.

3. Subject is willing and able to provide written informed consent prior to performing study procedures and be able to read and understand the study protocol.

4. Subject understands and agrees to comply with all planned study procedures.

5. Subject's right and left forearms must be free of large tattoos or skin abnormalities that would interfere with skin test administration and assessment in the opinion of the Investigator.

6. **For healthy uninfected subjects (Cohort 1):** Must have no history of signs or symptoms consistent with SARS-CoV-2 infection at any time within six months of enrollment AND in the Investigator's opinion, have no known intimate exposure or close contact to persons confirmed positive for SARS-CoV-2 infection.

7. **For recovered subjects (Cohort 2):** Must be > two months removed from confirmed diagnosis of SARS-CoV-2 infection independent of vaccination status.

8. **For subjects who have received a SARS-CoV-2 vaccine (Cohort 3):** must have received their final vaccination or booster dose at least four weeks prior to enrolment into the study with no known history of natural infection.

9. Subject receives a **negative SARS-CoV-2 PCR** test result at their screening or baseline visit.

8.3. Subject Exclusion Criteria

Subjects who meet one or more of the following criteria will not be considered eligible to participate in the clinical study:

Exclusion Criteria:

1. Female subjects who are pregnant or breastfeeding or planning to breastfeed at any time through 90 days after the Screening Visit.
2. Diagnosed with clinically significant and currently relevant cardiac disease:
    - Significant arrhythmia; heart block; heart failure; symptomatic coronary artery disease), recent myocardial infarction [within the past 2 years]) or QTcF >450 msec (male) or >470 msec (female).
3. Presence of erythema nodosum, eczema, psoriasis, or cellulitis.
4. Treatment with systemic corticosteroids, immunosuppressive drugs or any anti-viral treatment within two weeks prior to Screening.
5. History of immunosuppressive disease.
6. Prior adverse reaction to phlebotomy.
7. Prior adverse reaction to CANDIN® or similar material.
8. History of hypersensitivity to the study materials used for phlebotomy (i.e., latex, rubbing alcohol, cotton swabs, bandaids).
9. Documented allergic reaction to any of the excipients contained in the IP (e.g., polysorbate).
10. Subjects with dermatographism.
11. Subjects will be excluded if they have clinically significant underlying conditions associated with high risk for severe COVID-19 infections as identified by the Centers for Disease Control and Prevention (CDC) (Appendix 2). These conditions include, but are not limited to: chronic obstructive pulmonary disease, diabetes mellitus (Type 1 and 2), obesity, hypertension, heart disease, and cerebrovascular disease.

- Investigators should contact the Medical Monitor with questions regarding clinical significance of underlying conditions.

12. The subject has any clinically significant, uncontrolled, or unstable medical or surgical condition that could affect his or her ability to participate in the study or potentially compromise his or her well-being during the study.
13. Unable to understand the protocol requirements, instructions and study related restrictions, the nature, scope and possible consequences of the clinical study.
14. Healthy uninfected subjects who have any history of SARS-CoV-2 infection by clinical history or laboratory diagnosis (**Cohort 1 only**).

8.4. Restrictions

8.4.1. Medication

Medication restrictions applicable before dosing are described in Section 8.3 (exclusion criteria). Prior medication will be recorded.

Any medicinal product, prescribed or over-the-counter, taken by a subject other than the IPs, is considered concomitant medication. Any concomitant treatment will be given only if deemed strictly necessary by the Investigator or designee. Use of concomitant medication will be recorded and reported.

Subjects will be asked to report any vaccinations and/or boosters from time of study entry (signing of informed consent) until after study exit (early termination or completion of Visit 7, Day 180).

8.4.2. Contraception Rules

Female subjects are allowed to participate in the study if they are:

a) **Not of childbearing potential** defined as: post-menopausal (defined as at least 12 months natural spontaneous amenorrhea, or at least 6 weeks following surgical menopause), *or*

b) Naturally or surgically sterile (hysterectomy; bilateral oophorectomy; bilateral tubal ligation with surgery at least six weeks prior to study initiation).

c) Non-pregnant, non-lactating with negative pregnancy test at the Screening Visit and who use at least one of the following effective contraception options:

   i. Stable hormonal contraceptive for ≥ 90 days prior to screening and for at least seven days after final dose. If < 90 days prior to the study, additional use of one other effective contraception method until 90 days are reached is required, *or*

ii. Placement of an intrauterine device or intrauterine hormone-releasing system, *or*

iii. Use of double barrier methods of contraception (eg, male condom with diaphragm, male condom with cervical cap), *or*

iv. Successful male sterilization of the sole partner (subject must verbally confirm that appropriate post-vasectomy documentation of the absence of sperm in the ejaculate was provided after the procedure), *or*

v. True abstinence, when in line with the preferred and usual lifestyle of the subject.

Note: *Periodic abstinence, such as calendar, ovulation, symptothermal, post-ovulation methods, and withdrawal are not acceptable methods of contraception.*

8.5. Randomization Criteria

Not applicable as this will be an open-label study.

8.6. Subject Withdrawal Criteria

Subjects may withdraw from the study at any time at their own request without stating the reason(s) for withdrawal. A subject must be withdrawn by the Investigator (or by the Sponsor via the Investigator) from the study if one or more of the following occur:

- Withdrawal of consent by the subject.
- Withdrawal of subject by the Investigator or designee if inter-current illnesses occur that may invalidate the study data, if the subject was enrolled in violation of the study protocol, or if a significant study protocol violation occurred, at the discretion of the Investigator or designee.
- If discovered that the subject has entered the study in violation of the inclusion/exclusion criteria stated in the protocol.
- Critical protocol violation occurs during the study.
- Pregnancy during the treatment period.
- Lost to follow-up (i.e., following at least two telephone contact attempts and 1 certified letter).
- Investigator or the Sponsor stops the study, for any reason (e.g., suspension or discontinuation of study drug development).
- Investigator, medical monitor and/or Sponsor decision for any other reason not listed above that may jeopardise the health and/or safety of a subject or invalidate the results of the study.

A genuine effort must be made to determine the reason(s) why a subject fails to return for the necessary visits or is discontinued from the study. If the subject is unreachable by telephone, a registered letter, at the minimum, should be sent to the subject requesting him/her to contact the clinical unit.

8.6.1 Study Halting and Study Stopping Rules

Dosing may be halted temporarily to investigate before the entire study is terminated. If any of the below events occur, enrollment and dosing should be halted study-wide pending review by the Sponsor, Investigator, and/or Medical Monitor. Subjects enrolled in the study must continue to be followed for the duration of the study.

- One or more subjects with a serious adverse event (SAE) considered definitely, probably, or possibly related to the IPs.
- Three or more subjects with the same or similar grade 3 or higher AEs of the same type considered definitely, probably, or possibly related to IPs.
- One AESI, considered at least possibly related to the IPs.

If any of these events occur, enrollment and dosing should be paused study-wide pending review by the Sponsor, Investigator, and/or Medical Monitor. Subjects enrolled in the study must continue to be followed for the duration of the study.

9. TREATMENT OF SUBJECTS

9.1. Prior and Concomitant Medications

All prescription and over-the-counter medications used in the last three months before screening should be recorded at each visit (scheduled or unscheduled) in the case report form (CRF).

The use of corticosteroids, immunosuppressive drugs or any anti-viral treatment within two weeks prior to Screening is prohibited (see Section 8.3).

10. INVESTIGATIONAL PRODUCT

10.1. Identity of the Investigational Products

TNX-2100 skin test comprises of three distinct IPs named TNX-2110, TNX-2120 and TNX-2130 examined in this proposed POC study. These IPs fall into three distinct groupings based on the source:

- TNX-2110 – consists of 37 individual peptides which are representative of the dominant SARS-CoV T cell epitopes (Grifoni et al., 2020).
- TNX-2120 – consists of 98 individual peptides of approximately 20-mer with an approximate overlap of 10 amino acids. These peptides represent 1273 AA in SARS-CoV-2 spike (NCBI Reference Sequence: YP_009724390.1).
- TNX-2130 – consists of 16 individual peptides of approximately 20-mer with an approximate overlap of 10 amino acids. These peptides represent Orf3b (57 residue variant: Chan et al., 2020b; 57 and 22 residue variants: Konno et al., 2020) and Orf8 (Chan et al., 2020b)

Each IP contains 2.5 µg / mL per each peptide per vial (2 mL total volume per vial) in addition to compendial excipients. The IPs are provided as frozen liquid formulations containing phosphate buffer pH 7.0 with 0.31% polysorbate 20 and 4.6% mannitol. All IPs need to be thawed prior to use.

In addition, a "Diluent" is provided for a further manual dilution of the IPs. TNX-2100 "Diluent" is provided as a sterile solution consisting of phosphate buffer pH 7.0 with 0.31% polysorbate 20 and 4.6% mannitol.

All IPs will be manufactured under current Good Manufacturing Practices as sterile solutions for injection. All excipients are compendial. Refer to the current Investigational Brochure for composition details.

10.2. Study Drug Packaging, Labeling, and Storage

IPs will be packaged and labeled according to applicable local and regulatory requirements.

All supplies of IPs must be stored in accordance with the manufacturer's instructions.

The IPs will be stored in a securely locked area, accessible to authorized persons only, until needed for dosing.

All IPs shall be stored under the following conditions:

1. Long term storage. IP should remain frozen until ready to thaw on the day of Visit 1 (Day 1). Thawed IP must not be re-frozen.	-80˚C +/- 10˚C
2. After material is thawed (approximately 2-3 hours at ambient temperature) IP should be stored at 2 - 8°C until ready to administer. Thawed material should be discarded if not used within 8 hours on the day of thawing. Thawed IP must not be re-frozen.	2 - 8°C (upon thawing)

10.3. Preparation of Investigational Products

A total of 7 vials containing investigational drug product are involved in the 2-stage dilution and skin test administration process for each subject. Three (3) of the 7 vials are the 'undiluted' TNX-2110, TNX-2120 and TNX-2130 sterile solutions (2.5 µg/mL) for injection.

The remaining 4 vials are Diluent containing mannitol, polysorbate 20 and a phosphate buffer pH 7.0. Of the 4 Diluent vials, label 3 of them as '1:10 Dilution TNX-2110', '1:10 Dilution TNX-2120', and '1:10 Dilution TNX-2130' to represent the three "diluted" IPs. The remaining Diluent vial will be used as the negative control.

To prepare the "1:10 Dilution" solution, remove 222 µL from each of the "undiluted" vials of IP and add to the "1:10 Dilution" vial of the corresponding Diluent. Gently shake the solution for 2 minutes to ensure adequate mixing. Repeat the same process for each of the three IP.

Note: There is an 8th vial (in vivo positive control) involved in skin test administration, which is Nielsen Biosciences commercially available Candida albicans antigens (CANDIN®). Thus, is not considered an IP. Please refer to Section 11 and the Pharmacy Manual (Appendix 5) for further details.

10.4. Administration of the Investigational Products

In total, each subject will receive 8 intradermal injections (four injections per forearm), spaced two inches apart at pre-determined sites on the volar aspect of the forearms.

The three IPs (TNX-2110, TNX- 2120, TNX-2130) will be administered by intradermal injection (0.1 mL) in two concentration strengths ("1:10 dilution" and "Undiluted").

Subjects will also receive one intradermal injection of a positive control (CANDIN®), and one intradermal injection (0.1 mL) of a negative control "diluent". The three IPs (TNX-2110, TNX- 2120, TNX-2130) will be administered by intradermal injection (0.1 mL) in two concentration strengths ("1:10 dilution" and "undiluted").

Each vial containing IP, diluent, or CANDIN® will be limited to use of one subject. Remaining material will be discarded.

A detailed Pharmacy Manual outlining the IP administration and DTH assessment process will be provided to investigative site staff and is included in Appendix 5. A minimum of 2 members of the investigative site staff will be trained to administer the IPs and controls. Training materials, such as the CDC video for tuberculosis skin testing methodology[1], will be utilized.

Injections will be administered as shown in Figure 1 in two stages by the order and location illustrated in Table 4. In total, each subject will receive 8 intradermal injections, spaced two inches apart at pre-determined sites on the volar aspect of each forearm. Place injections approximately 3 inches from the antecubital crease.

In Stage 1 administration, subjects will receive the diluent (negative control), followed by the "1:10 dilution" strength of TNX-2110, TNX-2120, TNX-2130 in their left forearm, and CANDIN® (positive control) in their right forearm in the order and at specific locations indicated in Table 4 and the Pharmacy Manual (Appendix 5). Subjects will be monitored for local site reactions and systemic adverse reactions for 60 minutes. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. At 30 minutes post-administration, vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature) will be measured. If no systemic adverse reactions or unusual local site reactions per the Investigator's judgement are observed after 60 minutes, the subjects will proceed to Stage 2 administrations.

In Stage 2 administrations, subjects will receive the "undiluted" dose strength of TNX-2110, TNX-2120, TNX-2130 in the right forearm in the order and at specific locations indicated in Table 4 and the Pharmacy Manual (Appendix 5). After Stage 2 administrations, subjects will be monitored over the course of 30 minutes for unusual local site reactions and systemic adverse reactions. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. At 30 minutes post-administration, vital signs will be assessed. If no evidence of systemic adverse reactions or unusual local site reactions per the Investigator's judgement have occurred, subjects will be free to leave the clinic and will be

[1] https://tools.cdc.gov/podcasts/media/mp4/Mantoux_OC mp4

contacted 24 hours later for a brief telephone or in-person safety follow-up visit (Visit 2, Day 2;). Subjects will be instructed to keep the test sites clean, uncovered, and to not scratch or rub the area.

Figure 1 Standardized Intradermal Injection for the TNX-2100 Skin Test

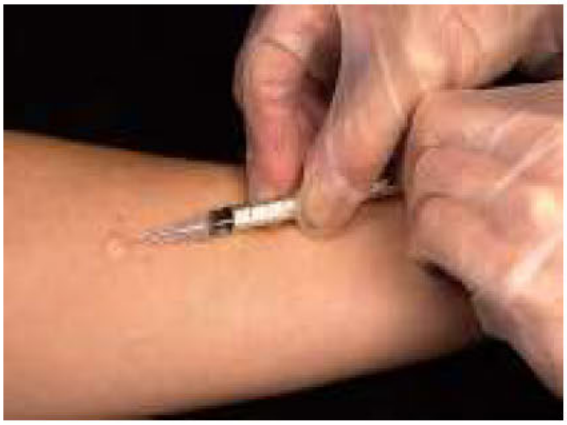


Table 4 Administration of In Vivo Investigational Products and Intradermal Controls

	To be administered in the numerical order shown	
	LEFT arm	***RIGHT arm***
Stage 1	① Negative control (0.1 mL diluent) IP at 'Diluted' dose[a] ② TNX-2110 ③ TNX-2120 ④ TNX-2130	⑤ Positive control (0.1 mL CANDIN®)
Safety Monitoring Period		
Stage 2		IP at 'Undiluted' dose[b] ⑥ TNX-2110 ⑦ TNX-2120 ⑧ TNX-2130
Safety Monitoring Period		

Note: the diluent will consist of phosphate buffer with 0.31% polysorbate 20 and 4.6% mannitol.
[a] 'Diluted' dose = 0.1 mL (0.025 µg peptide per 100 µL; concentration strength "1:10 dilution")
[b] 'Undiluted' dose = 0.1 mL (0.25 µg peptide per 100 µL; concentration strength "undiluted")
IP = Investigational Product

11. CONTROLS

Positive controls (Table 5) will used to confirm that study participants have intact T cell immunity and are not immunodeficient and include:

1. CANDIN® (Candida albicans Skin Test Antigen for Cellular Immunity) will be administered as an in vivo positive control to assess cell-mediated hypersensitivity to Candida albicans. Please see the Pharmacy Manual (Appendix 5) for further details.
2. A Tetanus Toxin Peptide Pool (TT) containing a pool of 326 overlapping peptides from a scan (15mers with 11 aa overlap) through Tetanus toxin (UniProt ID: P04958) of Clostridium tetani will be used as in vitro positive control for flow cytometry analysis
3. PepMix™ Candida (MP65) containing a pool of 92 peptides derived from a peptide scan (15mers with 11 aa overlap) through Mannoprotein MP65 (Swiss-Prot ID: Q9HEP1) of Candida albicans, will be used as in vitro control for flow cytometry analysis.

The negative control will contain ONLY the diluent (Table 5). The purpose of it is to rule out any irritant dermal response to cutaneous injection and will provide an indication of the size of the induration response to the negative control.

Table 5: Positive and Negative Controls for TNX-CA-C201 Study

Control	Composition	In Vitro/ In Vivo	Positive/ Negative Control	Purpose
Diluent	Phosphate buffer pH 7.0 with 0.31% polysorbate 20 and 4.6% mannitol	In Vitro *and* In Vivo	Negative	To rule out irritant dermal response to cutaneous injection
CANDIN®	Candida albican antigen	In Vivo	Positive	To confirm that study participants have intact T cell immunity and are not immunodeficient
Tetanus Toxin Peptide Pool[a]	Pool of 326 overlapping peptides from a scan (15mers with 11 aa overlap) through Tetanus toxin (UniProt ID: P04958) of Clostridium tetani (strain Massachusetts / E88) for T cell assays	In Vitro		

Control	Composition	In Vitro/ In Vivo	Positive/ Negative Control	Purpose
PepMix™ Candida [b]	Pool of 92 peptides derived from a peptide scan (15mers with 11 aa overlap) through Mannoprotein MP65 (Swiss-Prot ID: Q9HEP1) of Candida albicans (Yeast) for T cell assays.	In Vitro		

[a] https://www.peptides.de/2597/tetanus-toxin-peptide-pool
[b] https://shop.jpt.com/1-PepMix-trade-Peptide-Pools/6-Infectious-Disease-Antigens/10-PepMix-trade-Candida-MP65 html

11.1. Release of Clinical Study Supplies to the Investigator

The Sponsor or the Sponsor's designee's standard operating procedures for releasing clinical trial supplies to the site will be followed.

11.2. Study Drug Accountability and Reconciliation

The Investigator or designee is responsible for maintaining accurate accountability records of the IPs throughout the clinical study.

The Sponsor (or designee) will provide all study products. Study sites will acknowledge receipt of the IPs indicating shipment content and condition. IPs are not to be administered prior to confirmation from the Sponsor that the shipment temperatures were within range. Damaged supplies are not to be used and will be replaced. Accurate records of all IPs dispensed from and returned to the study site should be maintained by the site personnel. All unopened, expired, unused, partially used or empty materials must be accounted for and disposed, as directed by the Sponsor (or designee).

12. STUDY ASSESSMENTS AND PROCEDURES

12.1. Study Assessments

12.1.1. Demographics

Demographic information collected during the Baseline/Skin Test Administration Visit (Visit 1) will include the subject's age, gender, race and ethnicity as well as weight, height, and body mass index (BMI).

12.1.2. Medical History

A medical and allergy history including details regarding all illnesses, surgeries (to assess eligibility) must be obtained at Visit 1 and recorded in the CRF.

12.1.3. Previous and Concomitant Medications

All prescription and over-the-counter medications used in three months prior to Visit 1 should be recorded in the CRF.

12.1.4. Physical Examination

A complete physical examination will be performed at Visit 1. Rectal, breast, pelvic and/or urogenital examinations will not be performed. Height and weight are included in the Visit 1 physical examination.

12.1.5. Vital Signs

Vital signs (systolic and diastolic blood pressure, pulse, respiratory rate and body temperature) will be measured at Visit 1 prior to skin test administration. Vitals will also be collected after Stage 1 and Stage 2 skin test administrations, 30 minutes post-administration of IPs and controls.

12.1.6. Pregnancy Test

Women of childbearing potential (not menopausal, i.e., last period within the last year, or not surgically sterile) will have a urine pregnancy test performed at the Screening Visit (Visit 1) to determine/confirm their eligibility.

12.1.7. PCR Test for SARS-CoV-2

Nasopharyngeal samples will be collected from subjects to detect the presence or absence of SARS-CoV-2 viral RNA via PCR (reverse transcriptase [RT] or other) test. A laboratory PCR test or a sponsor approved rapid PCR (such as the Accula™ RT-PCR) test may be utilized. The test will be used as a screening tool. If a laboratory PCR test is used, subjects will complete a Screening Visit between Day -2 and Day -1 to allow processing of the sample prior to the Baseline/Skin Test

Administration Visit (Visit 1, Day 1). The rapid PCR test can be performed at Visit 1 (Day 1) and is preferred to facilitate enrolment by reducing the number of required site visits. A negative result must be reported before the subject can proceed to phlebotomy and skin test administration on Day 1.

12.1.8. Immunological Assessments

Blood samples for the immunological assessments (Table 6) will be collected as recommended by the Manufacturer. Samples will be collected at Visit 1 prior to IP administration.

Table 6: Immunological Assessments for TNX-CA-C201 Study

Assay	Purpose	Volume Per Subject	Blood Tube
Electrochemiluminescence Immunoassay	Serum for antibody testing	1.5 mL	Gel-barrier tube, red-top tube, or serum transfer tube, or plasma from lithium heparin tube, EDTA, or sodium citrate tube
ICS/Flow Cytometry	Whole blood for in vitro stimulation of PBMC's and characterization of Th1, Th2, Th17	40 mL	CPT 8 mL (heparin/ficoll/gel matrix) [×5]
T-Detect COVID test	Whole blood for detection of past SARS-CoV-2-specific T-cell immune response	4 mL	(2 mL) Lavender top (EDTA) tube [×2]

COVID = coronavirus disease; ECLIA = electrochemiluminescence immunoassay; EDTA = Ethylenediaminetetraacetic acid; ICS = intracellular cytokine staining; PBMC = Peripheral blood mononuclear cells; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; Th = T helper cell.

12.1.8.1. Electrochemiluminescence Immunoassay

Assessment of anti-SARS-CoV-2 antibody titers will be performed by FDA *E*mergency Use Authorized Electrochemiluminescence Immunoassay (ECLIA) for the detection and quantification of antibodies to SARS CoV-2. (LabCorp SARS-CoV-2 Semi-Quantitative Total Antibody, Spike TEST: 164090 CPT: 86769)

12.1.8.2. Flow Cytometric Analysis

Flow cytometric analysis of SARS-CoV-2-specific T-cell responses will be measured by stimulating PBMCs in vitro with test peptides, along with in vitro positive controls, JPT PepMix™ Candida (MP65) and a Tetanus Toxin Peptide Pool, and Diluent negative control.

12.1.8.3. Enzyme-linked Immune Assay

To characterize the Th17 response, IL-17 will be evaluated using an enzyme linked immune assay.

12.1.8.4. T-Detect COVID Test

The T-Detect COVID Test is a next generation sequencing based test to aid in identifying individuals with an adaptive T cell immune response to SARS-CoV-2.

The test analyzes DNA sequences from T cells to aid in identifying individuals with an adaptive T cell immune response to SARS-CoV-2, indicating recent or previous SARS-CoV-2 infection. A positive test result indicates recent or prior infection with SARS-CoV-2, while a negative test result indicates that a subject is unlikely to have been infected with SARS-CoV-2.

A 4 mL whole blood sample will be collected from which T cell receptor sequencing will be conducted using extracted genomic DNA.

12.1.9. Safety Laboratory Tests

The laboratory tests listed in Table 7 will be measured according to the study schedule in Table 3. The Investigator will review the laboratory results and judge values outside of their normal range as clinically significant or not clinically significant.

Table 7: Safety Laboratory Tests

Safety Laboratory Test	Descriptions
Serum Chemistry	Alanine aminotransferase (ALT/SGPT); albumin:globulin (A:G) ratio; albumin, serum; alkaline phosphatase, serum; aspartate aminotransferase (AST/SGOT); bilirubin, total; BUN; BUN:creatinine ratio; calcium, serum; carbon dioxide, total; chloride, serum; creatinine, serum; eGFR calculation; globulin, total; glucose, serum; potassium, serum; protein, total, serum; sodium, serum (LabCorp Comprehensive Metabolic Panel (14) TEST: 322000 CPT: 80053)
Hematology	Hematocrit; hemoglobin; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red cell distribution width (RDW); percentage and absolute differential counts; platelet count (RBC); red cell count; white blood cell count (WBC) (LabCorpComplete Blood Count With Differential, TEST: 005009 CPT: 85025)

12.1.10. Skin test results assessment

Investigational products are administered in two stages at Visit 1, as described in Section 10.4. In Stage 1 administration, subjects will receive the diluent (negative control), followed by the "1:10 dilution" strength of TNX-2110, TNX-2120, TNX-2130 in their left forearm, and CANDIN®

(positive control) in their right forearm. In Stage 2 administrations, subjects will receive the "undiluted" dose strength of TNX-2110, TNX-2120, TNX 2130 in the right forearm. The order and specific locations of injections are indicated in Table 4 and the Pharmacy Manual (Appendix 5). A ballpoint pen will be used to mark the arm but also describe precise location of injections (i.e., start three inches from antecubital crease).

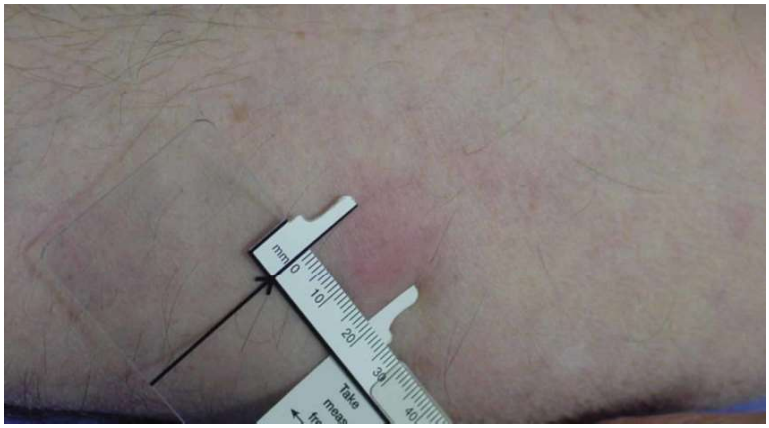
The area of induration will be measured at injection sites on the volar aspect of each forearm (Figure 2). To ensure standardization, calipers will be used to ensure accurate measurement of induration. A member of the study team will visually inspect injection site under good light and measure the induration (thickening of the skin), not erythema (reddening of the skin). Measurements will be made across two diameters. The mean of the longest and midpoint orthogonal diameters of the indurated area should be reported as the DTH response. For example, a reaction that is 10 mm (longest diameter) by 8 mm (orthogonal diameter) has a sum of 18 mm and a mean of 9 mm. The DTH response is therefore 9 mm. The diluent (negative control) is expected to elicit a negative skin test result (i.e., <5 mm), and the CANDIN (positive control) is expected to elicit a positive skin test result (i.e., ≥5 mm).

A digital caliper will be used to measure the area of induration as shown in above diagram. All the information (e.g., date and time of test administration, injection site location, lot number of peptide and measurement of area of induration) will be recorded in the CRF.

Further details regarding skin test administration and measurement are provided in the Pharmacy Manual (Appendix 5).

Photography of the injection site (volar forearms) after IP administration will be used for safety documentation of local skin reaction, such as redness and swelling, or unexpected findings such as rash, blistering and necrosis, which require assessment by the designated Investigator. Subject should lay arms flat, palms facing up. Site should photograph from above and in a well-lit area. Site should take photos uniformly for all subjects so that both forearms from inner elbow to wrist are visible.

Figure 2: Measurement of Area of Skin Induration



Ref: Ghosh et al, 2015.

12.1.11. Post-injection Safety Follow-up

Local (injection site) and systemic AEs will be evaluated 60 minutes post-Stage 1 administration and 30 min post-Stage 2 administration at Day 1 and at Visits 2, 3, 4 and 5; AEs will also be monitored at Visits 6 and 7. Subjects will also be asked to confirm that any areas of DTH reaction to skin test peptides (area of induration) have resolved. If in the unlikely event that visible evidence of the skin test exists on the subject's forearm at Visit 6 or 7, subject will be asked to return to the clinic for Investigator assessment at an unscheduled visit.

Local Adverse Events

The injection site should be visually inspected for local reactions and observed changes should be measured and recorded as AEs on the CRF page. Subjects should be asked about their general wellbeing (i.e., not prompted by specific questions regarding symptoms and side effects) and any reports of pain and/or itching or any unexpected symptoms should be evaluated and recorded in the CRF.

Local injection site skin reactions will be captured by subject self-reported 7-point Likert scales indicating severity of: erythema (redness), pain, tenderness, pruritis (itching), and swelling.

Photography of the injection site (volar forearms) after IP administration at Visit 1 and at Visit 3, 4, and 5, or unscheduled in person visit, will be used for safety documentation of local skin reaction, such as redness and swelling, or unexpected findings such as rash, blistering and necrosis, which require assessment by the designated Investigator.

For further information with regard to AEs, please refer to Section 14.

Systemic Adverse Events

Systemic adverse events will be assessed and graded using the toxicity scales defined in the FDA guidance for industry (FDA, 2007). Systemic adverse events will be graded using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening). Any AEs of systemic allergic reaction will be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (Appendix 1). Any reported or observed systemic AEs will be recorded on the systemic AE section of the CRF.

Medications and equipment (crash cart and adrenaline auto-injectors [EpiPen, Jext or Emerade]) to manage possible anaphylactic reactions will be available for immediate use. All investigative site staff will be trained in identifying anaphylaxis and a medically licensed professional trained in the clinical management of anaphylaxis will be present at Visit 1 for the duration of Stages 1 and 2.

12.2. Schedule of Study Procedures

The overall and detailed schedule for study procedures and visits is provided in Table 3.

12.2.1. Screening Visit (Day -2 to -1)

The Screening Visit is only required if a laboratory PCR test will be used to detect presence of active SARS-CoV-2 infection. The following procedures will be performed by the study personnel in the order given:

- Informed Consent: Before the potential subject has undergone any study-related screening procedures, the nature of the TNX-CA-C201 study, and the potential risks associated with it, will be explained to the subject, and the subject will be given an opportunity to ask questions to his or her satisfaction. After the questions are answered, but before proceeding further, the subject must read and sign a written ICF for protocol TNX-CA-C201. The signed ICFs will be retained in the Investigator's study file, and the date the subject signed the forms will be entered into the CRF. The subject will be provided with a copy of his or her signed and dated ICFs. The subject will be required to sign all updated informed consents.
- Perform nasopharyngeal swabbing for SARS-CoV-2 viral RNA detection via laboratory PCR test (note this result must be negative before proceeding to blood sample collection and skin test administration).

12.2.2. Visit 1 (Baseline/Skin Test Administration: Day 1)

The following procedures will be performed by study site personnel preferably in the order given:

- Informed Consent (if subject did not previously consent at a Screening Visit): Before the potential subject has undergone any study-related screening procedures, the nature of the TNX-CA-C201 study, and the potential risks associated with it, will be explained to the subject, and the subject will be given an opportunity to ask questions to his or her satisfaction. After the questions are answered, but before proceeding further, the subject must read and sign a written ICF for protocol TNX-CA-C201. The signed ICFs will be retained in the Investigator's study file, and the date the subject signed the forms will be entered into the CRF. The subject will be provided with a copy of his or her signed and dated ICFs. The subject will be required to sign all updated informed consents.

- Collect the medical history; if (based on the history) all of the inclusion and none of the exclusion criteria are fulfilled, continue with the following assessments.

- **Subjects in Cohort 2 only.** Data to be collected from each subject recovered from SARS-CoV-2 infection will include:
    - Information regarding where, when and what test was used to confirm SARS-CoV-2 infection.
    - Clinical symptoms/signs preceding SARS-CoV-2 diagnosis.
    - Time from onset of clinical signs of SARS-CoV-2.
    - Maximum SARS-CoV-2 severity experienced or contact.
    - Time from onset of exposure to person with SARS-CoV-2.
    - Therapies received for SARS-CoV-2.
    - Information regarding vaccination, if applicable.

- Determine that women of childbearing potential are using or will use highly effective method of contraception (i.e., double barrier method of contraception [e.g., intrauterine device and condom, spermicide and condom], a stable hormonal contraceptive for ≥90 days prior to the study or, if <90 days, additional use of a double barrier method until 90 days are reached, sexual abstinence or have a vasectomised partner until study completion).
- Review and document prior/current medications.
- Collect demographics. Data to be collected from each subject will include:
    - Age;
    - Sex;
    - Self-reported race and ethnicity;

  - Weight and height (BMI);
  - Underlying health conditions (if any);
  - Use of tobacco products or e-cigarettes (if any).

- Collect vital signs and ECG.
- Perform physical examination.
- Perform a urine pregnancy test for women of childbearing potential; do not test postmenopausal (≥1 year) or surgically sterile women.
- Perform nasopharyngeal swabbing for SARS-CoV-2 viral RNA detection via rapid PCR test (note: only required for subjects who did not have a laboratory PCR test at a Screening Visit between Day -2 and -1. Test result must be negative before proceeding to blood sample collection and skin test administration).
- Collect blood samples for:
  - T-Detect COVID Test;
  - ICS/Flow Cytometry;
  - SARS-CoV-2 antibody testing;
  - Comprehensive Metabolic Panel;
  - Complete blood count (CBC) with differential.
- Administer each of the IPs (peptides) 1:10 diluted dose first as outlined in Stage 1 of skin test administration.
- Adverse event monitoring. Subjects will be monitored for local site reactions and systemic adverse reactions for 60 minutes. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. Vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature) will be measured 30 minutes following administration of the "1:10 dilution" strength of TNX-2110, TNX-2120, TNX-2130, diluent (negative control) and CANDIN® (in vivo positive control). If no systemic adverse reactions or unusual local site reactions per the Investigator's judgement are observed after 60 minutes, the subjects will proceed to Stage 2 administration.
- Administration of IP (peptides) "undiluted" as outlined in Stage 2 (Section 12.1.10).
- Adverse event monitoring. Subjects will be monitored over the course of 30 minutes for unusual local site reactions and systemic adverse reactions. A photo of the injection sites (volar forearms) will be taken at the start of the post-administration safety monitoring period. At 30 minutes post-administration, vital signs will be assessed.

12.2.3. Visit 2 (Day 2)

Visit 2 will occur approximately 24 hours after administration of the intradermal injection of peptides. Subjects will have the option of an in person visit or a remote visit (i.e., telephone). Subjects will be asked if they have experienced any AEs or unusual skin reactions following administration of the IP.

If subjects opt for an in-person visit, photography of the injection sites (volar forearms) will be used for safety documentation of local site and any unexpected findings.

12.2.4. Visit 3 (Day 3)

The subject will return to the study site and the intradermal skin test will be read approximately 48 hours after administration of the intradermal injection of peptides. The basis of reading is the presence or absence of induration, which may be determined by inspection (from a side view against the light as well as by direct light) and by palpation.

The area of induration will be measured at injection sites on the volar forearms (Section 12.1.10). To ensure standardization, calipers will be used to ensure accurate measurement of induration. A member of the study team will visually inspect injection site under good light and measure the induration (thickening of the skin), not erythema (reddening of the skin). Measurements will be made across two diameters. The mean of the longest and midpoint orthogonal diameters of the indurated area should be reported as the DTH response.

The exact measurement of the induration in millimeters (mm) should be recorded in the subjects CRF.

Subjects will be asked if they have experienced any AEs or unusual skin reactions following administration of the investigational product. An AE occurring since the last visit will be documented in the subjects CRF.

Photography of the injection sites (volar forearms) will be used for safety documentation of local site and any unexpected findings.

12.2.5. Visit 4 (Day 4)

Visit 4 will occur in person approximately 72 hours after administration of the intradermal injection of peptides to conduct a brief medical review (general health inquiry) and to document AEs that occurred since the last study site visit.

The area of induration will be measured at injection sites on the volar aspect of both forearms.

Photography of the injection sites (volar forearms) will be used for safety documentation of local site and any unexpected findings.

12.2.6. Visit 5 (Day 5)

Visit 5 will occur in person approximately 96 hours after administration of the intradermal injection of peptides to conduct a brief medical review (general health inquiry) and to document AEs that occurred since the last study site visit.

The area of induration will be measured at injection sites on the volar aspect of each forearm.

Photography of the injection sites (volar forearms) will be used for safety documentation of local site and any unexpected findings.

12.2.7. Visit 6 (Day 30)

Visit 6 will occur by telephone approximately 30 days (720 hours) after administration of the intradermal injection of peptides to conduct a brief medical review (general health inquiry) and to document AEs that occurred since the last study site visit.

12.2.8. Visit 7 (Day 180)

Visit 7 will occur by telephone approximately 180 days after administration of the intradermal injection of peptides to conduct a brief medical review (general health inquiry) and to document AEs that occurred since the last study site visit.

13. STATISTICAL CONSIDERATIONS AND ANALYSES

Before database lock, a detailed statistical analysis plan (SAP) will be developed and finalized, which will include full description of the statistical analyses and the exact definition of variables to be analysed.

13.1. Populations for Analysis

The following analysis populations will be used during the study:

- Safety Population: includes any subjects in Cohort 1, 2, or 3 who receive at least 1 intradermal injection of the IPs.
- Intent-to-Treat (ITT) Population: includes any subjects in Cohort 1, 2, or 3 who provide written informed consent, satisfy all inclusion/exclusion criteria, and have a negative test result from PCR detection of SARS-CoV-2 viral RNA.
- Modified Intent-to-Treat (mITT) Population: includes subjects from ITT who complete stages 1 and 2 of administration and have induration responses for at least one post skin test administration visit at hour 48, 72, or 96.

13.2. General Considerations

Tabulations will be produced for appropriate demographic and assay parameters. For categorical variables, summary tabulations of the number and percentage within each category of the parameter will be presented. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented.

All descriptive statistical analyses will be performed using SAS statistical software (Version 9.4 or higher), unless otherwise noted. Medical History will be coded using Medical Dictionary for Regulatory Activities (MedDRA; dictionary Version 24.0).

13.3. Demographics

Demographic characteristics (age, sex, ethnicity and race) and anthropometric characteristics (height, weight and BMI) will be listed by subject and summarized by cohorts using descriptive statistics for all subjects in the safety population.

- Age (years) at screening as continuous summary
- Sex
- Ethnicity
- Race

- Weight
- Height
- BMI

13.4. Subject Disposition

The disposition of subjects who were enrolled (i.e., signed the ICF) in the study as well as reasons and the time period of discontinuation from the study and from treatment will be summarised for each treatment group (and overall).

13.5. Safety Analyses

The safety of intradermally administered TNX-2100 SARS-CoV-2 peptides will be measured as detailed in Section 7.2.1. All safety analyses will be conducted using the Safety Population.

13.5.1. Adverse Events

All AEs will be listed. The number and percent of subjects experiencing an event will be tabulated for each System Organ Class and Preferred Term. The AEs will also be tabulated according to severity and causality.

For this study, AESIs are anaphylactic reactions and enhanced disease from repeated exposure to SARS-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

Serious AEs (SAE) will be listed separately.

13.5.2. Clinical Laboratory Tests

Individual data listings of laboratory results will be presented for each subject. Flags will be attached to values outside of the laboratory's reference limits along with the Investigator's assessment. Clinically significant laboratory test abnormalities that were considered AEs by the Investigator will be presented in the AE listings.

Clinical laboratory tests (observed values) will be summarized descriptively in tabular format. Shift tables will be presented for select laboratory parameters.

13.5.3. Vital Signs

Individual data listings of vital signs (observed and change from baseline) will be presented for each subject. Individual clinically significant vital signs findings that were considered AEs by the Investigator will be presented in the AE listings.

Observed values as well as change from baseline data will be summarized descriptively in tabular format.

13.5.4. Physical Examination

Abnormal physical examination findings will be listed.

13.6. Efficacy Analyses

The efficacy analyses include primary, secondary, and exploratory endpoints as outlined in Section 7.2.2. Efficacy analyses will be conducted using the mITT population.

The frequency and percentage of subjects with a maximal area of induration of ≥5 mm will be summarized within each cohort and by overall subjects.

Cytokine and cell surface T-cell phenotype markers will be summarized descriptively within each cohort and by overall subjects.

Enzyme linked immune assay parameters will be summarized descriptively by study visit within each cohort and by overall subjects.

Results obtained from subjects deemed to be T-cell-specific immunodeficient will be collected, documented and reported, but will not be included in the analysis of the efficacy of the assay to detect a DTH response to SARS-CoV-2 peptides.

13.7. Interim Analysis

The skin test DTH result assessments are obtained at Visits 3, 4 and 5 (48, 72 and 96 hours post administration of skin test). An interim analysis will be conducted after all subjects have completed Visit 5 (or early terminated prior to Visit 5), and a database lock of the efficacy and safety data up to Visit 5 will occur. The purpose of the interim analysis will be to assess the presence of DTH reactions in response to intradermal injection of synthesized TNX-2100 SARS-CoV-2 peptide antigens, and to assess safety up to 96 hours post-administration. A full database lock will occur after all subjects have completed Visit 7 (or early terminated prior to Visit 7).

14. DEFINITIONS, RECORDING, AND REPORTING OF ADVERSE EVENTS

14.1 Definition of Adverse Events

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

A treatment-emergent adverse event (TEAE) is an AE that either commenced following initiation of study treatment or was present prior to study treatment but increased in frequency or severity following initiation of study treatment.

Other untoward events occurring in the framework of a clinical study will be recorded as AEs, e.g., those occurring during treatment-free periods (including Screening or post-treatment follow-up periods; see the Schedule of Activities, Table 3) in association with study-related procedures and assessments, or under placebo. For study drugs, lack of efficacy may be an expected potential outcome and should not be reported as an AE unless the event is unusual in some way, e.g., greater in severity.

Concomitant illnesses, which existed prior to entry into the clinical study, will not be considered AEs unless they worsen during the treatment period. Pre-existing conditions will be recorded as part of the subject's medical history.

14.2 Adverse Event Recording

14.2.1 Coding the Adverse Event

Standard medical terminology should be used in describing AEs. MedDRA Version 24.0 will be used as the standard coding dictionary for AEs and in describing the subject's medical history, and the World Health Organization (WHO) Drug Dictionary (B3 Global VMAR 2021) will be used to code concomitant medications. Informal descriptions should be avoided.

14.2.2 Severity of Adverse Event

The severity of an AE refers to the extent of clinical abnormalities and to the extent of which an AE affects the subject's daily activities (Table 8, FDA Guidance for Industry; Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007). The term "severity" is used to describe the intensity of an event. This is not the same as "serious". Seriousness, not severity, serves as the guide for defining regulatory reporting

obligations. The highest severity grade attained should be reported, for AEs with divergent severities.

Severity will be categorized according to event category (local, systemic, other).

14.2.3 Relationship of Adverse Events to Study Drug

The Investigator will assess the potential relationship of the AE to study drug using the following descriptions.

- ***Not Related:*** This category applies to an AE that is clearly not related to the study drug beyond a reasonable doubt. That is, another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the administration of study drug and/or a causal relationship is considered biologically implausible.
- ***Unlikely Related:*** This category applies to an AE that could reasonably be considered caused by something else, and where there is no known or expected response pattern to the suspected study drug.
- ***Possibly Related:*** This category applies to an AE that follows a reasonable temporal sequence from administration of the study drug and that follows a known or expected response pattern to the suspected study drug, but that could readily have been produced by a number of other factors.
- ***Probably Related:*** This category applies to an AE that follows a reasonable temporal sequence from administration of study drug and that follows a known or expected response pattern to the suspected study drug. The AE could have been produced by a number of other factors, but the alternatives are not likely.
- ***Definitely Related:*** This category applies to an AE that is clearly related to study drug. The AE follows a clear temporal sequence from administration of study drug, follows a known or expected response pattern to the study drug, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure and that could not be reasonably explained by the known characteristics of the subject's clinical state.

14.2.4 Local Adverse Event by 7-point Likert Scale

The subject self-reported severity of local AEs will be graded by the subject using a 7-point Likert scale. The scale will assess the subjects' evaluation of severity of erythema (redness), pain, pruritis and swelling following the intradermal injection.

Full details can be found in Appendix 3.

14.2.5 Toxicity Assessment Scales

Clinically significant symptoms and other adverse events will be assessed and graded by the Investigator as defined in the FDA guidance for industry: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" using a 4-point system (Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, and Grade 4 = potentially life threatening) (Table 8).

Table 8: Tables for Clinical Abnormalities (FDA, 2007)

Vital Signs *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
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	ER visit or hospitalization for hypotensive shock
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	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Local AEs will be graded as described in Section 14.2.4. Adverse events of systemic allergic reaction will be graded according to the World Allergy Organization Grading Scale for Systemic Allergic Reactions (see Appendix 1).

14.2.6 Adverse Events of Special Interest

In consideration of the limited safety data available on prior skin test studies, two specific AESIs have been identified:

- Anaphylactic reaction (Appendix 4).
- Enhanced disease from repeated exposure to SARS-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

Adverse events of special interest are events of scientific and medical interest specific to the further understanding of the TNX-2100 skin test. Investigators are advised to monitor subjects carefully for potential development of these adverse events as well as other adverse events (eg. local site reaction) and are required to have the appropriate safety equipment on site (crash cart and adrenaline auto-injectors [EpiPen, Jext or Emerade]). All investigative site staff will be trained in

identifying anaphylaxis and a medically licensed professional trained in the clinical management of anaphylaxis will be present at the skin test administration visit (Visit 1, Day 1) for the duration of Stages 1 and 2. Investigators are required to communicate such events immediately to the Sponsor. An AESI can be serious or non-serious. All AESIs should be entered in the Electronic Data Capture (EDC) within 24 hours of reported onset and will be recorded in the CRF. Serious AESIs will be recorded and reported as per Section 14.3.1.

14.3 Serious Adverse Events and Serious Adverse Drug Reactions

A serious adverse event (SAE; experience) or reaction is any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening;
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect, or;
- Is an important medical event.

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

14.3.1 Reporting of Serious Adverse Events

The investigative site is required to report any SAE within 24 hours of becoming aware of the event, directly to Premier PV using the contact information provided in the protocol. Premier PV will confirm the receipt of any formally submitted information regarding a reportable event to the sender within one business day by email. Premier Pharmacovigilance will document and archive the appropriate receipt confirmation sent to the sender.

Events that are subject to immediate notification (within 24 hours of knowledge by any investigational site personnel) include the following:

- Serious AE (whether or not deemed drug-related or expected);
- AESI as specified in the protocol, i.e., anaphylactic reaction, and enhanced disease from repeated exposure to SARS-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

  Note an AESI can be serious or non-serious. (see Section 14.2.6).

- Pregnancy.

Any SAE that occurs at any time during the study, including a clinically significantly abnormal laboratory test result that is considered serious, must be reported to the Sponsor or its designee(s) so that the Sponsor may comply with regulatory obligations. If the SAE is life-threatening or fatal, it must be reported to the Sponsor or its designee(s) immediately, by facsimile and telephone. For these and all other SAEs, an SAE report form must be completed and sent by facsimile or email to the Sponsor or its designee(s) within 1 working day of the site's initial awareness of the event. These requirements apply equally to all subjects, regardless of the study phase or the at-risk subject's treatment assignment or dosage.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room, anaphylaxis requiring respiratory support, blistering, rash, or necrosis.

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

Any death occurring during the study, during the per-protocol follow-up period, or reported to the Investigator after study participation (no required post-study time limit) must be reported to the Sponsor or its designee(s) immediately, whether or not it is considered treatment-related.

If any SAE occurs in the course of the study, then Investigators or other site personnel inform the Premier PV team and Tonix Medical Monitor **within 24 hours of becoming aware of the event.**

Initial SAE reports must be followed by detailed descriptions. These should include copies of hospital case records and other documents when requested. Telephone and e-mail reports must be confirmed promptly either by facsimile or by email.

For reporting SAEs, the Sponsor's designated Medical Monitor should be called, and the relevant forms submitted to Premier Pharmacovigilance Department within 24 hours of the site's awareness of the SAE.

The contact information for the Medical Monitor is as follows:

[redacted]

The Investigator, or the Sponsor or designee in the case of a central Institutional Review Board (IRB), also must notify the IRB of the occurrence of the SAE, in writing, as soon as is practicable and in accordance with local law. A copy of this notification must be provided to the Sponsor or its designee.

In the event of an SAE that meets the criteria for expedited reporting, an investigational new drug (IND) Safety Report will be prepared for submission to the FDA. Emergency unblinding is not applicable as this is an open label study.

14.4 Pregnancy

Subjects found to be pregnant at the Baseline/Skin Test Administration Visit will be discontinued from study participation prior to drawing blood samples and administering IP.

Subjects who become pregnant within 30 days of IP administration should be discontinued from the study and followed until birth or any pregnancy termination. If a pregnancy is reported within the 30 days, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy. Abnormal pregnancy outcomes (e.g., spontaneous abortion beyond first trimester, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

If a pregnancy is reported during the study, the investigator should send email the SAE Pregnancy Report Form to [redacted] within 1 day of learning of the pregnancy. If e-mail is unsuccessful, [redacted]

15 ETHICAL CONSIDERATIONS

15.2 Ethical Conduct of the Study

This protocol is written in accordance with the principles established by the 18th World Medical Assembly General Assembly (Helsinki, 1964) and amendments and clarifications adopted by subsequent General Assemblies. The Investigator will make sure that the study described in this protocol is conducted in full conformance with those principles, the protocol, current FDA regulations, ICH Good Clinical Practices (GCP) guidelines, Good Laboratory Practices (GLP) guidelines, local ethical and regulatory requirements, including the Federal Food, Drug and Cosmetic Act, US applicable Code of Federal Regulations (title 21), any EC requirements relative to clinical studies. As required by the US FDA, the study drug may not be shipped to any participating Investigator until the requisite study documentation has been submitted to the Investigational New Drug Application.

Should a conflict arise, the Investigator will follow whichever law or guideline affords the greater protection to the individual subject. The Investigator will also make sure he or she is thoroughly familiar with the appropriate administration and potential risks of administration of the study drug, as described in this protocol and the Investigator's Brochure, prior to the initiation of the study.

15.3 Institutional Review Board

The IRB must be a properly constituted board or committee operating in accordance with 21 CFR Part 56, "Institutional Review Boards." This protocol, any protocol amendments, the associated informed consent forms, and the informed consent procedures must be submitted to the IRB for review and approved before the enrollment of any subject into the trial.

All types of subject recruitment or advertising information must be submitted to the Sponsor or its designee and to the IRB for review and approval prior to implementation. IRB approval of any protocol amendments must be received before any of the changes outlined in the amendments are put into effect, except when the amendment has been enacted to eliminate a potential hazard to study subjects. In such cases, the chair of the IRB should be notified immediately and the amendment forwarded to the IRB for review and approval.

15.4 Written Informed Consent

It is the responsibility of the Investigator to obtain signed written informed consent from each potential study subject prior to the conduct of any screening or other study procedures. This written informed consent will be obtained after the methods, objectives, and potential risks of the study have been fully explained to the potential subject. The Investigator must explain to each subject that he or she is completely free to refuse to enter the study or to withdraw from it at any time.

The subject should also be asked in the ICF for permission for the Investigator or his/her designee to contact the subject's other personal physicians, as appropriate, concerning participation in the study.

The method of obtaining and documenting informed consent and the contents of the ICF will comply with ICH GCP guidelines, the requirements of 21 CFR Part 50, "Protection of Human Subjects", the Health Insurance Portability and Accountability Act (HIPAA) regulations, and all other applicable regulatory requirements. A properly executed written ICF shall be read, signed, and dated by each subject prior to entering the trial or prior to performing any study procedure. The original signed and dated ICF will be kept on file at the study site. Subjects will be given a copy of the signed ICF and will be informed of any new developments during the course of the study that might influence their continued participation in the study.

The Investigator or a qualified designee will be available to answer each subject's questions throughout the study, and all questions must be answered to the subject's satisfaction. If the protocol is amended and a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IRB, prior to subsequently obtaining each subject's consent.

Receipt of written informed consent will be documented in each subject's or potential subject's CRF. The signed ICF must remain in each subject's study file and must be available for verification by study monitors at all times.

16 DATA HANDLING, RECORD KEEPING, MONITORING AND AUDITS

16.2 Maintaining Privacy and Confidentiality

In order to maintain subject privacy, all CRFs, study drug accountability records, and other documents, including communications between the study site and the Sponsor, will identify subjects only by their initials and their assigned study identification numbers. If required, the Investigator will grant monitors and auditors from the Sponsor or its designee and/or regulatory authority's access to subjects' original medical records for verification of the data gathered on the CRFs and to audit the data collection process. Subjects' confidentiality will be maintained and will not be made publicly available unless mandated by applicable laws and regulations.

16.3 Maintaining Essential Clinical Documents

Study site files for the retention of regulatory documents will be established at the beginning of the study, maintained for the duration of the study, and retained according to FDA and ICH/GCP guidelines and applicable regulatory requirements. The records maintained must be adequate to fully document appropriate protection of study subjects, the validity of the study, the integrity of the data, and the manner in which the study was conducted.

The Investigator's site file, copies of protocols, CRFs, originals of test result reports, drug disposition logs, correspondence, records of written informed consent, and other documents pertaining to the conduct of the study must be kept on file by the Investigator and in readily accessible order for at least 2 years after the last approval of a marketing application, until at least 2 years have elapsed after formal discontinuation of the clinical development of the investigational product, or according to local regulatory requirements. No study document may be destroyed without prior written consent from the Sponsor or its designee. Should the Investigator wish to withdraw from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing in advance if a custodial change is to occur. It is important that the Investigator remain ready to provide background information from the archived study records on request.

The Sponsor or designee will maintain adequate study records for at least two years after the last approval of a marketing application or for at least two years after clinical development of the study drug for the indication being studied has been discontinued. After that period, the sponsor will be contacted to determine whether the study records will be forwarded to the Sponsor, destroyed, or kept at the location of the designee or another facility for a longer period of time.

16.4 Data Handling

Unless otherwise specified, procedures, data collection and evaluation will be conducted as per the Standard Operating Procedures of the contract research organization (CRO). The Investigator will assume the responsibility of ensuring the completeness and accuracy of the clinical data. All data will be verified for quality control and will also be subject to audits from the Sponsor or designee to ensure quality.

All laboratory results will be analyzed by an accredited and licensed clinical laboratory facility. Clinical laboratory data will be transferred from the central laboratory to the clinical database maintained by the CRO using systems which are validated and Part 11-compliant.

The responsible clinical study monitor(s) will check data at the monitoring visits to the clinical study site. The Investigator will ensure that the data collected are accurate, complete, and legible. Any changes made to the clinical data will be documented with a full audit trail.

Aspects of the clinical and statistical phases of the study, including all associated documentation may be reviewed by the Quality Assurance Unit of the contract research organization using a risk-assessment approach. The final clinical and statistical report will be audited to ensure that, as far as can be reasonably established, the methods described and the results reported accurately reflect the raw data generated during the study.

16.5 Case Report Forms

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study subject. Data must be recorded on CRFs approved by the Sponsor or its designee. Data (including AEs) will be recorded on raw data sheets and/or electronic or paper source documents.

If selected data is collected via paper (subject questionnaires, etc.), the data must be entered into the CRF and verified that it has been transcribed correctly.

16.6 Clinical Laboratory Certification

A central clinical laboratory will be used to analyze all samples in this study. The Investigator must maintain, on file, written evidence that the central clinical laboratory to be used is certified under the Clinical Laboratory Improvement Act or equivalent certification (depending on local regulations). Further, the Investigator will maintain a copy of the certification, the range of normal values, the effective dates for the ranges, and the units of measurement for all laboratory tests requested in the protocol. If any of the laboratory measurements will be transformed and/or categorized in any way, a description of the procedures(s) used should be included. The Investigator is expected to receive these documents before the shipment of clinical supplies.

16.7 Site Monitoring and the Right to Review Records

Monitoring and auditing procedures developed by the Sponsor and/or its designee will be implemented to ensure compliance with FDA and ICH GCP and GLP guidelines.

The Sponsor's designated representative (the monitor or auditor) will contact the Investigator and conduct regular visits to the clinical site. In extenuating circumstances related to the COVID-19 pandemic, remote monitoring will be permissible. The monitor will be expected and allowed to verify the Investigator's qualifications, to inspect clinical site facilities, and to inspect study records, including proof of IRB review, with the stipulation that subjects confidentiality will be maintained in accordance with local and federal regulations, including HIPAA requirements. The monitor will also be responsible for confirming adherence to the study protocol, inspecting CRFs and source documents, and ensuring the integrity of the data. CRFs will be checked for accuracy, completeness, and clarity and will be cross-checked for consistency with source documents, including laboratory test reports and other subject records. Instances of missing or uninterpretable data will be resolved in coordination with the Investigator.

The monitor/auditor will also investigate any questions concerning adherence to regulatory requirements. Any administrative concerns will be clarified and followed. The monitor will maintain contact with the site through frequent direct communications with the study site by e-mail, telephone, facsimile, and mail. The Investigator and all other site personnel agree to cooperate fully with the monitor and will work in good faith with the monitor to resolve any and all questions raised and difficulties detected by the monitor.

16.8 Audits and Inspections

The Investigator understands that regulatory authorities, the IRB, and/or the Sponsor or their designees have the right to access all CRFs, source documents, and other study documentation for on-site audit or inspection and will retain this right from the start of the study to at least two years after the last approval of a marketing application or for at least two years after clinical development of the study drug for the indication being studied has been discontinued. The Investigator is required to guarantee access to these documents and to cooperate with and support such audits and inspections.

17 CONFIDENTIALITY

17.2 Protection of Subject Anonymity

The Investigator must make sure that each subject's anonymity is maintained. On CRFs or other documents submitted to the Sponsor or its agent, subjects should not be identified by their names, but rather by their initials and the assigned study identification numbers. The Investigator should keep a separate record of the subject initials, randomization codes, subject names, address, and contact information. Documents that contain the names associated with these initials and codes are not for submission to the Sponsor or its agents (e.g., written informed consent forms). These records should be maintained by the Investigator in strict confidence except to the extent necessary to allow auditing by regulatory authorities, the Sponsor, or its agents. These records should be kept in compliance with HIPAA regulations.

17.3 Confidentiality of Study Information

All information relevant to this study, whether supplied by the Sponsor or its agents to the Investigator or collected by the Investigator in support of this study, is privileged and confidential. The Investigator agrees to use this information to carry out the study and will not use it for other purposes without written consent from the Sponsor. It is understood that the Investigator is under obligation to provide the Sponsor with all data obtained during the study. The information obtained from this study will be used by the Sponsor towards the clinical development of the indicated investigational drug and may be disclosed by the Sponsor to regulatory authorities, other Investigators, corporate partners, or consultants as required.

17.4 Publication of Data and Protection of Trade Secrets

No presentations, abstracts (including meeting abstracts), or other publications based on the conduct or results of this study will be permitted without the express written permission of the Sponsor or its designated agent. All such presentations or publications will proceed only as collaborations between the Sponsor and the Investigators.

If the Investigator wishes to publish the results of this study, a copy of the proposed manuscript or abstract (including meeting abstracts) will be provided to the Sponsor or its designee for review, revision, and approval at least sixty (60) days before the expected date of submission for publication, unless otherwise arranged with the Sponsor in writing. This will enable the Sponsor to protect its proprietary information and augment the publication with insights or information of which the Investigator may not be aware.

Subject names and other identifiers, such as photographs or audio or video recordings, may not be disclosed in any publication or public forum without prior written authorization from the subjects involved or their legal guardians.

18 LIST OF REFERENCES

Black CA. Delayed type hypersensitivity: current theories with an historic perspective. Dermatol Online J. 1999 May;5(1):7. PMID: 10673450.

Barrios, Y., Franco, A., Sanchez-Machin, I., Poza-Guedes, P., Gonzalez-Perez, R., & Matheu, V. (2021a). A novel application of delayed-type hypersensitivity reaction to measure cellular immune response in SARS-CoV-2 exposed individuals. *Clinical Immunology*, 226, 108730.

Barrios, Y., Franco, A., Sánchez-Machín, I., Poza-Guedes, P., González-Pérez, R., & Matheu, V. (2021b). The Beauty of Simplicity: Delayed-Type Hypersensitivity Reaction to Measure Cellular Immune Responses in RNA-SARS-CoV-2 Vaccinated Individuals. *Vaccines*, 9(6), 575.

Chan KW, Wong VT, Tang SCW. COVID-19: An Update on the Epidemiological, Clinical, Preventive and Therapeutic Evidence and Guidelines of Integrative Chinese-Western Medicine for the Management of 2019 Novel Coronavirus Disease. *Am J Chin Med*. 2020a;48(3):737-762. doi:10.1142/S0192415X20500378.

Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect*. 2020(b) Jan 28;9(1):221-236. doi: 10.1080/22221751.2020.1719902. Erratum in: Emerg Microbes Infect. 2020 Dec;9(1):540. PMID: 31987001; PMCID: PMC7067204.

Chen J, Qi T, Liu L, et al. Clinical progression of patients with COVID-19 in Shanghai, China. *J Infect*. 2020;80(5):e1-e6. doi:10.1016/j.jinf.2020.03.004.

Cox LS, Sanchez-Borges M, Lockey RF. World Allergy Organization Systemic Allergic Reaction Grading System: Is a Modification Needed? J Allergy Clin Immunol Pract. 2017 Jan-Feb;5(1):58-62. e5. doi: 10.1016/j.jaip.2016.11.009. PMID: 28065342.

FDA. Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. 2007. Available at https://www.fda.gov/media/73679/download.

Ghosh, P. & Bhattacharjee, Debotosh & Nasipuri, Mita. (2015). Automation in Sputum microscopy: A Hybrid Intelligent Techniques in Diagnostic Device Automation. IGI Global.

Grifoni A, Sidney J, Zhang Y, et al. A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. *Cell Host Microbe.* 2020;27(4):671-680.e2. doi:10.1016/j.chom.2020.03.002.

Guan W.J., Ni Z.Y., Hu Y. Clinical characteristics of coronavirus disease 2019 in China [published online ahead of print, 2020 Feb 28] *N Engl J Med*. 2020 doi: 10.1056/NEJMoa2002032. NEJMoa2002032.

Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in Lancet. 2020 Jan 30;:]. *Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5.

Landsteiner K, Chase MW. Studies on the sensitization of animals with simple chemical compounds : vii. Skin sensitization by intraperitoneal injections. *J Exp Med*. 1940;71(2):237-245. doi:10.1084/jem.71.2.237.

Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. 2020. 10.1056/NEJMoa2001316.

Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020 Feb 22;395(10224):565-574. doi: 10.1016/S0140-6736(20)30251-8. Epub 2020 Jan 30. PMID: 32007145; PMCID: PMC7159086.

Konno Y, Kimura I, Uriu K, Fukushi M, Irie T, Koyanagi Y, Sauter D, Gifford RJ; USFQ-COVID19 Consortium, Nakagawa S, Sato K. SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant. *Cell Rep*. 2020 Sep 22;32(12):108185. doi: 10.1016/j.celrep.2020.108185. Epub 2020 Sep 4. PMID: 32941788; PMCID: PMC7473339.

Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity*. 2020;52(6):971-977.e3. doi:10.1016/j.immuni.2020.04.023.

People with Certain Medical Conditions. cdc.gov. Updated October 14, 2021. Accessed December 7, 2021. https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html.

Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report-Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium. *J Allergy Clin Immunol* 2006;117(2):391- 7.

Surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]. NCBI Reference Sequence: YP_009724390.1.

Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang Y-Q, Wang Q, Miao H. Lymphopenia predicts disease severity of COVID- 19: a descriptive and predictive study. *Signal Transduct Target Ther*. 2020; 5:33.

Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069. doi:10.1001/jama.2020.1585

Wen, W., Su, W., Tang, H. et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. *Cell Discov* 6, 31 (2020). https://doi.org/10.1038/s41421-020-0168-9.

Wölfel, R., Corman, V.M., Guggemos, W. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 581, 465–469 (2020). https://doi.org/10.1038/s41586-020-2196-x.

Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;323(13):1239-1242. doi:10.1001/jama.2020.2648.

Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*. 2020 Feb 20;382(8):727-733. doi: 10.1056/NEJMoa2001017. Epub 2020 Jan 24. PMID: 31978945; PMCID: PMC7092803.

Zweiman, B. Cell-mediated immunity in health and disease. Allergy Principles and Practices, Mosby, Saint Louis, Chapter 50: 696, 1998.

19 APPENDICES

APPENDIX 1. WORLD ALLERGY ORGANIZATION GRADING SCALE FOR SYSTEMIC ALLERGIC REACTIONS

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptom(s)/sign(s) of 1 organ system present[a] **Cutaneous** Generalized pruritus, urticaria, flushing, or sensation of heat or warmth[b] **or** Angioedema (not laryngeal, tongue, or uvular) **or** **Upper respiratory** Rhinitis – (eg, sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) **or** Throat-clearing (itchy throat) **or** Cough perceived to originate in the upper airway, not the lung, larynx, or trachea **or** **Conjunctival** Erythema, pruritus or tearing **Other** Nausea, metallic taste, or headache	***Symptom(s)/sign(s) of more than 1 organ system present*** **or** **Lower Respiratory** Asthma, cough, wheezing, shortness of breath (eg, less than 40% PEF or FEV_1 drop, responding to an inhaled bronchodilator) **or** **Gastrointestinal** Abdominal cramps, vomiting, or diarrhea **or** **Other** Uterine cramps	**Lower Respiratory** Asthma (eg, 40% PEF or FEV1 drop NOT responding to an inhaled bronchodilator) **or** **Upper Respiratory** Laryngeal, uvula, or tongue edema with or without stridor	**Lower or upper respiratory** Respiratory failure with or without loss of consciousness **or** **Cardiovascular** Hypotension with or without loss of consciousness	Death

Patients may also have a feeling of impending doom, especially in grades 2, 3, or 4.

Note: Children with anaphylaxis seldom convey a sense of impending doom and their behavior changes may be a sign of anaphylaxis; eg, becoming very quiet or irritable and cranky.

Scoring includes a suffix that denotes if and when epinephrine is or is not administered in relationship to onset of symptom(s)/sign(s) of the SR: a, ≤5 minutes; b, >5 minutes to ≤10 minutes; c, >10 minutes to ≤20 minutes; d, >20 minutes; z, epinephrine not administered.

The final grade of the reaction will not be determined until the event is over, regardless of the medication administered. The final report should include the first symptom(s)/sign(s) and the time of onset after the subcutaneous allergen immunotherapy injection[c] and a suffix reflecting if and when epinephrine was or was not administered, **eg, Grade 2a; rhinitis: 10 minutes.**

Final Report: Grade a-d, or z_____ First symptom(s)/sign(s)_____ **Time of onset of first symptom_____**
Comments[d]
[a] Each grade is based on organ system involved and severity. Organ systems are defined as cutaneous, conjunctival, upper respiratory, lower respiratory, gastrointestinal, cardiovascular, and other. A reaction from a single organ system such as cutaneous, conjunctival, or upper respiratory, but not asthma, gastrointestinal, or cardiovascular is classified as a grade 1. Symptom(s)/sign(s) from more than one organ system or asthma, gastrointestinal, or cardiovascular are classified as grades 2 or 3. Respiratory failure or hypotension with or without loss of consciousness define grade 4 and death grade 5. The grade is determined by the physician's clinical judgement. [b] This constellation of symptoms may rapidly progress to a more severe reaction. [c] Symptoms occurring within the first minutes after the injection may be a sign of severe anaphylaxis. Mild symptoms may progress rapidly to severe anaphylaxis and death. [d] If signs or symptoms are not included in the table or the differentiation between a SR and vasovagal (vasodepressor) reaction, which may occur with any medical intervention, is difficult, please include comments, as appropriate.

Source: Cox et al., 2017

APPENDIX 2. CDC UNDERLYING MEDICAL CONDITIONS ASSOCIATED WITH HIGHER RISK FOR SEVERE COVID-19

From the Centers for Disease Control and Prevention (CDC) COVID-19 Information for People with Certain Medical Conditions (https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html) dated 14 October 2021.

This list is presented in alphabetical order and **not in order of risk.**

CDC completed an evidence review process for each medical condition on this list to ensure they met criteria for inclusion on this list. CDC conducts ongoing reviews of additional underlying condition and some of these conditions might have enough evidence to be added to the list.

As we are learning more about COVID-19 every day, this list **does not** include all medical conditions that place a person at higher risk of severe illness from COVID-19. Rare medical conditions, including many conditions that primarily affect children, may not be included below. The list will be updated as the science evolves.

A person with a condition that is not listed may still be at greater risk of severe illness from COVID-19 than people of similar age who do not have the condition and **should talk with their healthcare provider**.

Medical Conditions

1. Cancer
    a. Having cancer can make you more likely to get severely ill from COVID-19. Treatments for many types of cancer can weaken your body's ability to fight off disease. At this time, based on available studies, having a history of cancer may increase your risk.
2. Chronic kidney disease
    a. Having chronic kidney disease of any stage can make you more likely to get severely ill from COVID-19.
3. Chronic liver disease
    a. Having chronic liver disease, such as alcohol-related liver disease, non-alcoholic fatty liver disease, and autoimmune hepatitis, and especially cirrhosis, or scarring of the liver, can make you more likely to get severely ill from COVID-19.

4. Chronic lung diseases
    a. Having chronic lung diseases can make you more likely to get severely ill from COVID-19. These chronic lung diseases may include:
        i. Asthma, if it's moderate to severe
        ii. Bronchiectasis (thickening of the lungs airways)
        iii. Bronchopulmonary dysplasia (chronic lung disease affecting newborns)
        iv. Chronic obstructive pulmonary disease (COPD), including emphysema and chronic bronchitis
        v. Having damaged or scarred lung tissue such as interstitial lung disease (including idiopathic pulmonary fibrosis)
        vi. Cystic fibrosis, with or without lung or other solid organ transplant
        vii. Pulmonary embolism (blood clot in the lungs)
        viii. Pulmonary hypertension (high blood pressure in the lungs)
5. Dementia or other neurological conditions
    a. Having neurological conditions, such as dementia, can make you more likely to get severely ill from COVID-19.
6. Diabetes (type 1 or type 2)
    a. Having either type 1 or type 2 diabetes can make you more likely to get severely ill from COVID-19.
7. Down syndrome
    a. Having Down syndrome can make you more likely to get severely ill from COVID-19.
8. Heart conditions
    a. Having heart conditions such as heart failure, coronary artery disease, cardiomyopathies, and possibly high blood pressure (hypertension) can make you more likely to get severely ill from COVID-19.
9. HIV infection
    a. Having HIV (Human Immunodeficiency Virus) can make you more likely to get severely ill.
10. Immunocompromised state (weakened immune system)
    a. Having a weakened immune system can make you more likely to get severely ill from COVID-19. Many conditions and treatments can cause a person to be immunocompromised or have a weakened immune system. Primary

immunodeficiency is caused by genetic defects that can be inherited. Prolonged use of corticosteroids or other immune weakening medicines can lead to secondary or acquired immunodeficiency.

11. Mental health conditions
    a. Having mood disorders, including depression, and schizophrenia spectrum disorders can make you more likely to get severely ill from COVID-19.
12. Overweight and obesity
    a. Overweight (defined as a body mass index (BMI) $\geq$ 25 kg/m^2 but < 30 kg/m^2), obesity (BMI $\geq$ 30 kg/m^2 but < 40 kg/m^2), or severe obesity (BMI of $\geq$ 40 kg/m^2), can make you more likely to get severely ill from COVID-19. The risk of severe COVID-19 illness increases sharply with
13. Pregnancy
    a. Pregnant and recently pregnant people (for at least 42 days following end of pregnancy) are more likely to get severely ill from COVID-19 compared with non-pregnant people.
14. Sickle cell disease or thalassemia
    a. Having hemoglobin blood disorders like sickle cell disease (SCD) or thalassemia can make you more likely to get severely ill from COVID-19.
15. Smoking, current or former
    a. Being a current or former cigarette smoker can make you more likely to get severely ill from COVID-19. If you currently smoke, quit. If you used to smoke, don’t start again. If you’ve never smoked, don’t start.
16. Solid organ or blood stem cell transplant
    a. Having had a solid organ or blood stem cell transplant, which includes bone marrow transplants, can make you more likely to get severely ill from COVID-19.
17. Stroke or cerebrovascular disease, which affects blood flow to the brain
    a. Having cerebrovascular disease, such as having a stroke, can make you more likely to get severely ill from COVID-19.
18. Substance use disorders
    a. Having a substance use disorder (such as alcohol, opioid, or cocaine use disorder) can make you more likely to get severely ill from COVID-19.
19. Tuberculosis
    a. Having tuberculosis can make you more likely to get severely ill from COVID-19.

APPENDIX 3. 7-POINT LIKERT SCALE

The 7-point Likert scale is self-reported by the subject after the context of the scale, described below, is either read to them, or alternatively, it is read by the subjects themselves.

Please take a few moments to think about the symptoms you have had due to your DTH Skin Test in the (specified time period). It is very important for us to get this information. We will be using it as one of the ways to identify the severity of local skin reactions.

According to the scale below, please indicate the severity of erythema, pain, pruritis and swelling following the intradermal injection over the (specified time period).

1. No problem.
2. Minimal problem (can be easily ignored without effort).
3. Mild problem (can be easily ignored with effort).
4. Moderate problem (cannot be ignored but does not influence my daily activities).
5. Moderately severe problem (cannot be ignored and occasionally limits my daily activities.
6. Severe problem (cannot be ignored and often limits my concentration on daily activities).
7. Very severe problem (cannot be ignored and markedly limits my daily activities and often requires rest).

APPENDIX 4. CLINICAL CRITERIA FOR DIAGNOSING ANAPHYLAXIS

As detailed by Sampson et al., (2006), anaphylaxis is broadly defined as "a serious allergic reaction that is rapid in onset and may cause death." Diagnostic criteria defined by the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network during the second symposium on the definition and management of anaphylaxis, modified from Sampson et al. are as follows:

Anaphylaxis is highly likely when any of the following three criteria is fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula) and at least one of the following:
    a. respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow, hypoxemia).
    b. reduced blood pressure or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence).
2. Two or more of the following that occur rapidly after exposure to a likely allergen for that subject (minutes to several hours):
    a. involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
    b. respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow, hypoxemia)
    c. reduced blood pressure or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
    d. persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting).
3. Reduced blood pressure after exposure to known allergen for that subject (minutes to several hours):
    a. adult: systolic blood pressure <90 mm Hg or >30% decrease from that person's baseline.

In the event of suspected anaphylaxis or severe hypersensitivity, vital signs, including oxygen saturation and respiration rate, will be measured. Other assessments will be performed at the

discretion of the Investigator. As a precaution, each investigational site should have a resuscitation cart nearby.

APPENDIX 5. PHARMACY MANUAL