Statistical Analysis Plan

Sponsor	Tonix Pharmaceuticals, Inc.
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"
Protocol Number:	TNX-CA-C201
Document Version:	1

Approvals

[redacted]

Note: The date of the analysis plan is the date of the final approval signature.

Document Revision History

Revision History			
SAP Version #	**Modification**	**Description and Rationale**	**Superseded SAP Version #**
1.0	N/A	N/A	N/A

Table of Contents

List of Abbreviations

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of special interest
BMI	Body mass index
CANDIN®	Candida albicans skin test antigen for cellular hypersensitivity
COVID-19	Coronavirus disease 2019
CRF	Case report form
DTH	Delayed-type hypersensitivity
ELISpot	Enzyme-linked immune absorbent spot
FDA	Food and Drug Administration
GCP	Good clinical practices
GLP	Good laboratory practices
ICF	Informed consent
ICH	International Council for Harmonisation
ICS	Intracellural cytokine staining
IP	Investigational product
IRB	Institutional review board
MedDRA	Medical Dictionary for Regulatory Activities
NP	Nasopharyngeal
PBMC	Peripheral blood mononuclear cells
PI	Principal investigator

Abbreviation	Definition
POC	Proof-of-Concept
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System Organ Class
SAS	Statistical Analysis System
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
Th	T helper cell
TEAE	Treatment-emergent adverse event
WHO	World Health Organization

1. Overview

This statistical analysis plan (SAP) describes the planned analysis and reporting for Tonix Pharmaceuticals, Inc. protocol number TNX-CA-C201 (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"), dated 07-Dec-2021, version Final v2.0, amendment 01. Reference materials for this statistical plan include the protocol and the accompanying sample data collection documents. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analysis.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials (ICH, 1998). All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association (ASA, 2018) and the Royal Statistical Society (RSS, 2014), for statistical practice.

The planned analyses identified in this SAP may be included in clinical study reports (CSRs), regulatory submissions, or future manuscripts. Also, post-hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc or unplanned, exploratory analysis performed will be clearly identified as such in the final CSR.

The final clinical database cannot be locked until the final SAP has been approved and signed.

2. Study Objectives and Endpoints

2.1. Study Objectives

2.1.1. Primary Objective

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

2.1.2. Secondary Objectives

The secondary objectives are:

- To estimate sensitivity and specificity of test as a marker of active or 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.

2.2. Study Endpoints

2.2.1. Efficacy Endpoints

2.2.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint of this study is the 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.

2.2.1.2. Secondary Efficacy Endpoints

The secondary efficacy endpoints of this study include the following:

- 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.

2.2.1.3. Exploratory Efficacy Endpoint

The exploratory efficacy endpoint is a characterization of Th17 response by enzyme linked immune assay.

2.2.2. Safety Endpoints

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

- Incidence of AEs.
    - Clinically abnormal 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” 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.
    - 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 SAR-CoV-2 (in subjects who have recovered from SARS-CoV-2 infection or in subjects recently vaccinated against SARS-CoV-2).

2.3. Modified Evaluation

Because the outcome of area of induration for the DTH reactions was not as anticipated, the DTH reactions will not be statistically analyzed. Any primary, secondary, or exploratory efficacy endpoint will be omitted from the analysis and only safety will be assessed.

3. Overall Study Design and Plan

3.1. Overall Design

This will be 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 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 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. The estimated study duration will be approximately 6 months. 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.

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.

3.2. Sample Size and Power

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. Approximately 90 subjects (30 subjects per cohort) will be enrolled.

3.3. Study Population

The study population consists of male and female adults, aged 18 to 65 years of age, inclusive. Subjects will be uninfected/unexposed healthy individuals (cohort 1), SARS-CoV-2 convalescent subjects (cohort 2), or SARS-CoV-2 vaccinated subjects (cohort 3).

3.4. Treatments Administered

Intradermal injections will be administered in two stages. At Stage 1, each subject will receive the diluent (negative control), followed by the "1:10 dilution" strength of TNX-2110, TNX 2120 and TNX 2130 in their left forearm, and a CANDIN® (positive control) in their right forearm. If no systemic adverse reactions or unusual local site reactions per the Principal Investigator's (PI) judgement are observed during 60 minutes after Stage 1 administration, the subjects will proceed to Stage 2.

At 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. If no evidence of systemic adverse reactions or unusual local site reactions, per the PI's judgement, have occurred during the 30 minutes post-administration, subjects will be free to leave the clinic. Subjects will be instructed to keep the injection sites clean, uncovered, and to not scratch or rub the area.

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

3.5. Method of Assigning Subjects to Treatment Groups

Subjects will be enrolled in parallel into 3 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 enrollment 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 enrollment into the study no known history of natural infection.

3.6. Blinding and Unblinding

This will be an open-label study.

3.7. Schedule of Events

The full schedule of activities can be found in Table 3 of the protocol.

4. Statistical Analysis and Reporting

4.1. Introduction

Data processing and tabulation of descriptive statistics will use SAS (release 9.4 or higher), unless otherwise noted. If the use of other software is warranted, the final statistical methodology report will detail what software was used for what purposes.

Continuous (quantitative) variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, minimum, and maximum.

Categorical (qualitative) variable summaries will include the frequency and percentage of subjects who are in the particular category or each possible value. In general, the denominator for the percentage calculation will be based upon the total number of subjects in the study population for the cohorts, or other describing characteristic, unless otherwise specified. The denominator for by-visit displays will be the number of subjects in the relevant study population with non-missing data at each visit.

The minimum and maximum will be reported with the same degree of precision (i.e., the same number of decimal places) as the observed data. Measures of location (mean and median) will be reported to 1 degree of precision more than the observed data and measures of spread (SD) will be reported to 2 degrees of precision more than the observed data.

Percentages will be presented to 1 decimal place, unless otherwise specified.

5. 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, 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.

- 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.

6. Analysis Populations

The following analysis populations are planned for this study:

- **Safety Population**: includes any subjects in Cohort 1, 2, or 3 who receive at least 1 intradermal injection of the IPs. This population will be used for all safety tables.

- **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 RT-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.
- **Enrolled Subjects**: includes subjects who have been consented and screened, with eligibility verified. This population will be used for all listings.

7. General Issues for Statistical Analysis

7.1. Statistical Definitions and Algorithms

7.1.1. Baseline

The last observation recorded before the first intradermal injection of the IPs will be used as the baseline observation for all calculations of change from baseline.

7.1.2. Adjustments for Covariates

Not applicable to this study.

7.1.3. Multiple Comparisons

There will be no adjustments for multiple comparisons.

7.1.4. Handling of Dropouts or Missing Data

Any subject who withdraws from the study will not be replaced.

Unless specified otherwise in any of the previous sections, missing data will not be imputed.

7.1.5. Analysis Visit Windows

Data will be summarized by nominal study visit recorded in the database.

For tabulated summaries, only the scheduled visits will be included. Unscheduled assessments will be listed.

7.1.6. Derived Variables

- Change from baseline = value at current time point – value at baseline.

- TEAE = any adverse event with an onset date/time after the first intradermal injection of the IPs.

7.1.7. Data Adjustments/Handling/Conventions

Adverse events will be coded using the MedDRA version 24.0 thesaurus.

A treatment related AE is any AE with a relationship to the study drug as judged by the investigator.

For partial start dates:

- If the year is unknown, then do not impute the date but instead assign a missing value.
- If the year is known, but the month or month and day is unknown, then:
    - If the year matches the year of first dose date and the end date (if present) is after first dose date, or the AE/medication is ongoing, then impute as the month and day of the first dose date. If this produces a date after the end date, assign 01 January.
    - Otherwise, assign 01 January.
- If the year and month are known, but the day is unknown, then:
    - If the month and year match the month and year of the first dose date, then impute as the day of the first dose date. If this produces a date after the AE/medication end date, assign 01.
    - Otherwise, assign 01.

For partial end dates:

- If the year is unknown, then do not impute the date but assign as missing value.
- If the year is known but the month or month and day is unknown, then:
    - If the year matches the year of the last date of the study (date of last contact if subject lost to follow-up; date of completion or early termination otherwise), then impute as the month and day of the last date of the study.
    - Otherwise, assign 31 December.
- If the year and month are known, but day is unknown, then:
    - If the month and year match the month and year of the last date of the study, then impute as the day of the last date of the study.
    - Otherwise, assign the last day of the month.

These conventions will be applied only to adverse events.

Missing times will be not be imputed, they will be left as missing.

8. Study Patients/Subjects and Demographics

8.1. Disposition of Patients/Subjects

Subject disposition information will be listed. Disposition for all subjects will include tabulations of the number of subjects enrolled, completing, and withdrawing, along with reasons for withdrawal, for each cohort (and overall).

8.2. Protocol Violations and Deviations

Protocol deviations will be listed.

8.3. Demographics and Other Baseline Characteristics

Summary statistics for age, sex, ethnicity, race, height, weight, and BMI will be summarized by cohorts for subjects in the Safety population. For the continuous variables, the number of non-missing values and the mean, standard deviation, minimum, median, and maximum will be tabulated. For the categorical variables, the counts and proportions of each value will be tabulated.

Demographic and anthropometric variables (age, sex, ethnicity, race, height, weight, and BMI) will be listed by subject. In addition, medical history, smoking history, and vaccination history will be listed by subject.

8.4. Exposure and Compliance

IP exposure and dosing information will be listed for each subject.

8.5. DTH Responses

The DTH responses will be listed for each subject by visit.

9. Safety Analysis

Tables for the safety analyses will be conducted on the Safety population, which includes any subjects in Cohort 1, 2, or 3 who receive at least 1 intradermal injection of the IPs. Listings will include enrolled subjects.

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

9.1. Adverse Events

Treatment emergent AEs are defined as AEs 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. Adverse events of special interest (AESI) include 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).

All AEs occurring after completion of the informed consent process and before the end of study, regardless of relationship to study drug, will be included and classified by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) v24.0.

For treatment-emergent AEs (TEAEs), the following will be summarized and presented for the Safety population:

An overall summary of TEAEs, which includes:

a. the number and percentage of patients experiencing a TEAE
b. the number and percentage of patients experiencing a TEAE by strongest relationship to IP
c. the number and percentage of patients experiencing a TEAE by highest severity grade
d. the number and percentage of patients experiencing a systemic allergic reaction by highest grade according to the World Allergy Organization Grading Scale for Systemic Allergy Reactions
e. the number and percentage of patients experiencing a treatment emergent SAE (TESAE)
f. the number and percentage of patients experiencing a TEAE leading to study discontinuation
g. the number and percentage of patients experiencing a treatment emergent AE of Special Interest

In the overall summary of TEAEs table, besides tabulating the number and percentage of patients, the total number of TEAE episodes will also be provided. If a patient has repeated episodes of a particular TEAE, all episodes will be counted in the summary table.

All occurrences of all AEs will be listed for each patient. The listing will contain the following information: verbatim term, SOC, PT, severity (Grade1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, and Grade 4 = Potentially Life Threatening), World Allergy Organization Grading Scale for Systemic Allergic Reactions (Grades 1-5) [where applicable], relationship to study medication, date/time and day of onset, date/time and day of resolution, the outcome, whether the event was life-threatening, fatal, or a TEAE, SAE, or AESI, if it requires or prolongs hospitalization, persistent or significant disability/incapacity, congenital anomaly or birth defect,

or important medical event. Listings will be sorted by patient identification number, onset date, SOC, and PT. If the onset date is completely missing, these events will be presented first. If the onset date is missing a month or a day, these events will be presented before any complete dates. AEs with a relationship to study drug of possibly related will be considered related.

Local skin reaction AEs will be listed by 7-point Likert scales indicating severity of the events:

- 1 = No problem;
- 2 = Minimal problem (can be easily ignored without effort);
- 3 = Mild problem (can be 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).

All collected data will be presented in listings.

9.2. Clinical Laboratory Evaluations

Individual data listings of laboratory results will be presented for each subject. Flags will be attached to the value outside of the laboratory's reference limits.

9.3. Vital Signs

Individual data listings of vital signs (observed and change from baseline) will be presented for each subject.

9.4. Physical Examination

Abnormal physical examination findings will be listed.

9.5. 12-Lead Electrocardiogram

12-Lead electrocardiogram interpretations will be listed.

9.6. Concomitant Medication

Prior and concomitant medications will be listed. Medications that started before initial intradermal injections of investigational products will be considered prior medications whether or not they were stopped before initial intradermal injections of investigational products. Any

medications continuing or starting after initial intradermal injections of investigational products will be considered to be concomitant. If a medication starts before initial intradermal injections of investigational products and continues after, it will be considered both prior and concomitant.

Medications will be coded using World Organization Drug Dictionary Anatomical Therapeutic Chemical classes and preferred terms.

10. Changes from Planned Analysis

Because the outcome of area of induration for the DTH reactions was not as anticipated, the DTH reactions will not be statistically analyzed. Any primary, secondary, or exploratory efficacy endpoint will be omitted from the analysis and only safety will be assessed.

11. Other Planned Analysis

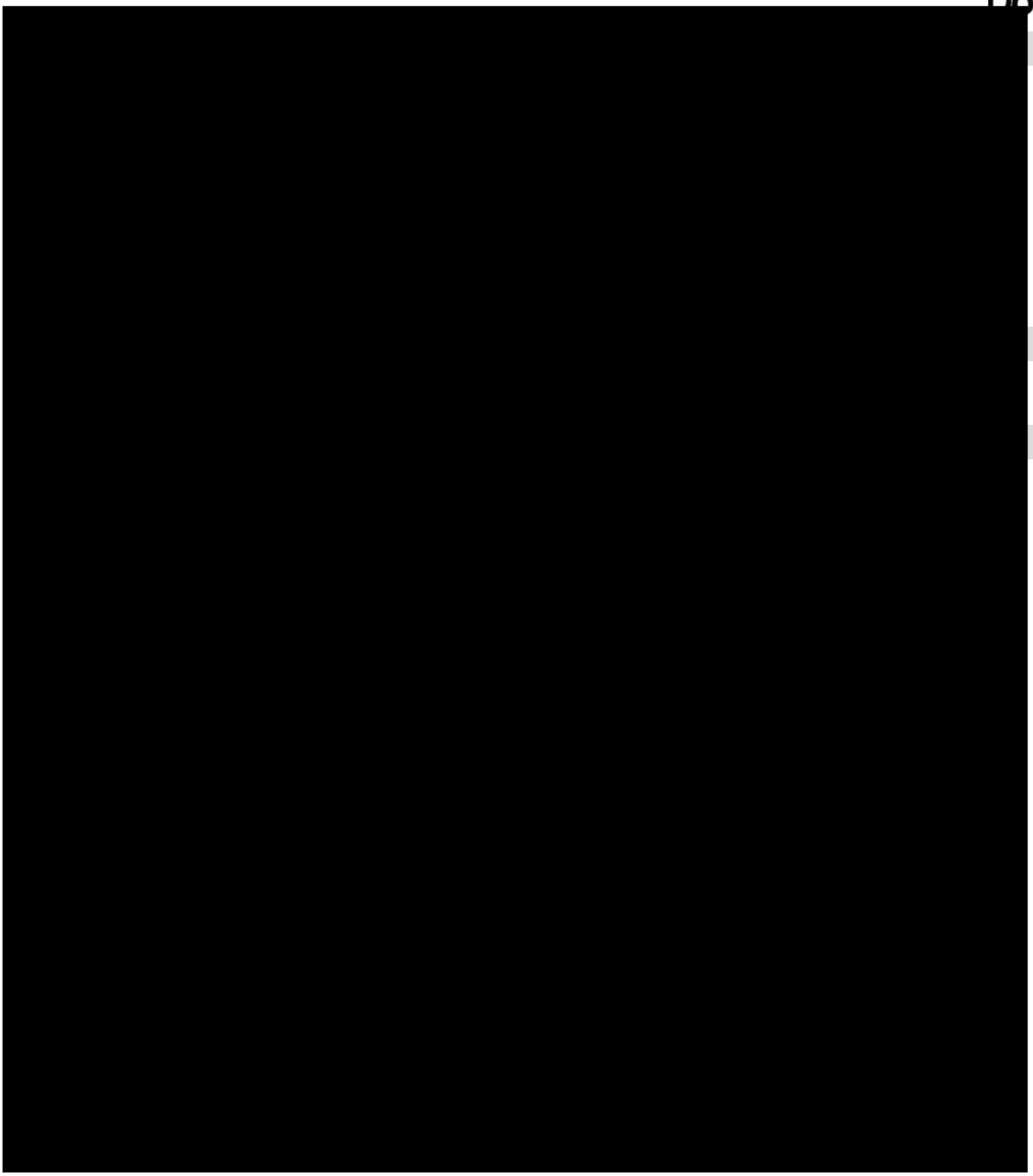
Not applicable for this study.

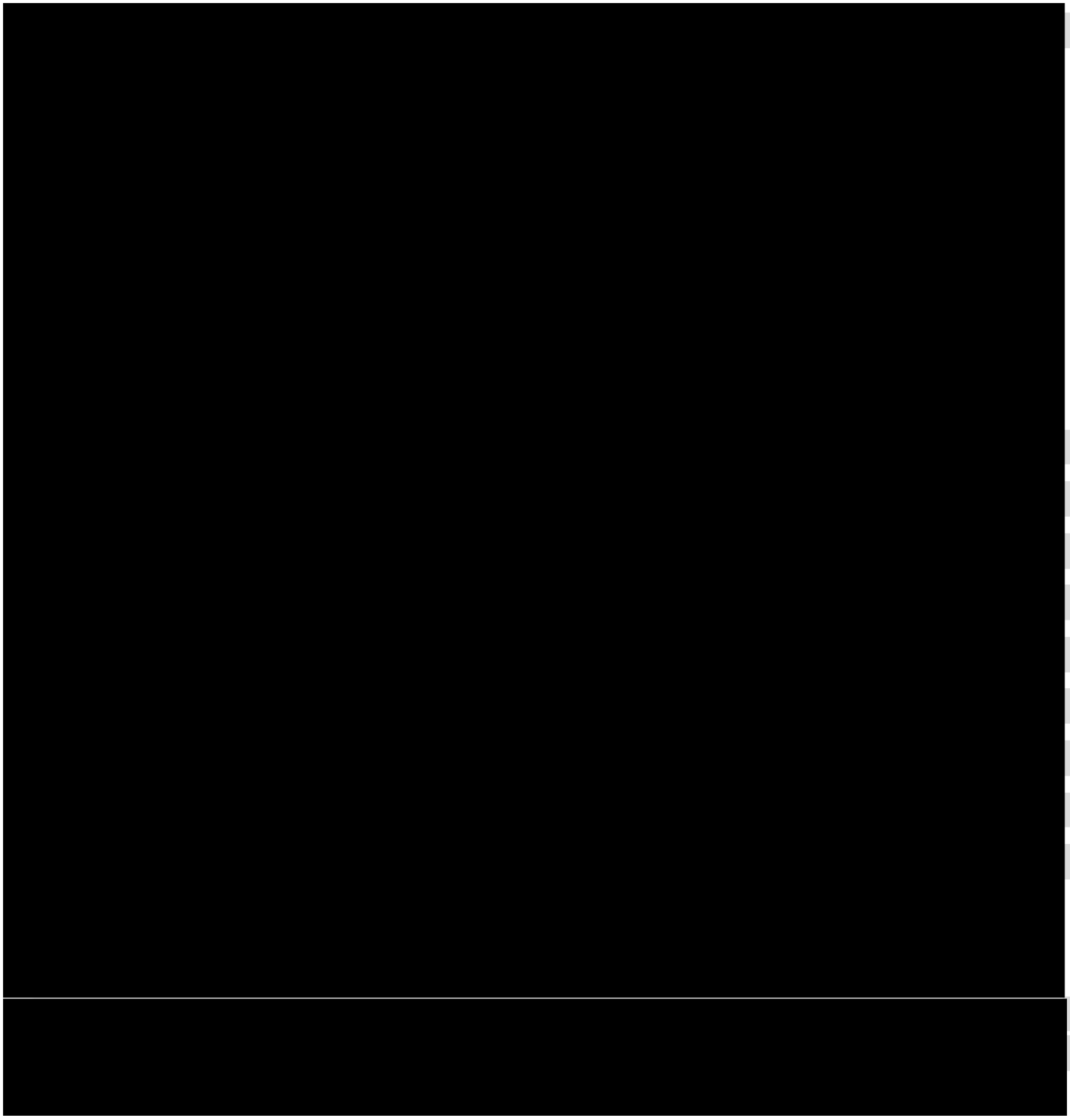
12. References

ASA. (2018) Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, April 2018. http://www.amstat.org/about/ethicalguidelines.cfm

ICH (1998). ICH Harmonised Tripartite Guideline. Statistical Principles for Clinical Trials E9; 1998. https://database.ich.org/sites/default/files/E9_Guideline.pdf

RSS. (2014) The Royal Statistical Society: Code of Conduct, 2014. https://rss.org.uk/about/policy-and-guidelines/code-of-conduct/.





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