Statistical Analysis Plan

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Trial: A Phase 1 Clinical Trial of a Peptide-Based Group A Streptococcal Vaccine

Trial Registration: ClinicalTrials.gov Identifier: NCT04882514

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Abbreviations

Abbreviation	Definition

Section 1: Administrative Information

1.1 SAP revisions

Protocol version	Updated SAP version no.	Section number changed	Description of and reason for change	Date changed

1.2 Roles and responsibility

5 Names, affiliations, and roles of SAP contributors

Contributor	Affiliation	Role of Contributor
Dr. Maryna Yaskina	WCHRI	Co-author
Dr. Vanessa Meier-Stephenson	Dept of Medicine	Co-author
Kelly Kim	Clinical Trials Office	Co-author

1.3 Signatures

Author of "A Phase 1 Clinical Trial of a Peptide-Based Group A Streptococcal Vaccine Study" SAP:

Name (print):	Dr. Maryna Yaskina
Signature:	M. Yaskina
Date (dd-mmm-yyyy):	June 27, 2025

Principal Investigator of the "A Phase 1 Clinical Trial of a Peptide-Based Group A Streptococcal Vaccine Study":

Name (print):	Dr. Vanessa Meier-Stephenson
Signature:	
Date (dd-mmm-yyyy):	15Jul2025

Section 2: Introduction

2.1 Background and rationale

Group A streptococci (Strep A) or Streptococcus pyogenes is a major human pathogen causing an estimated 500,000 deaths worldwide each year. Disease can range from mild pharyngitis to more severe infections, such as necrotizing fasciitis, septicemia, and toxic shock syndrome. Untreated, Strep A infection can lead to the serious post streptococcal pathologies of rheumatic fever/rheumatic heart disease and post-streptococcal glomerulonephritis. An effective vaccine against Strep A would have great benefits worldwide. Here, we test two products, J8 and p*17—both peptide derivatives of a highly conserved region in the M protein, in combination with the protein subunit K4S2 of SpyCEP, an IL-8 protease associated with neutrophil chemoattraction. Each peptide is individually conjugated to cross reacting material (CRM197), and the conjugated peptide vaccines are abbreviated as J8-K4S2 or p*17-K4S2.

2.2 Objectives

Primary objective is safety, whereby we monitor and document possible adverse events via

(1) Clinical signs and symptoms
(2) Standard laboratory parameters (hematological and biochemical).
(3) Echocardiogram (mitral regurgitation).

Secondary objectives are immunogenicity, where we have three measures*:

(1) Antibody titers before and after 1, 2, and 3 doses of Strep A combination peptide vaccines and compare titers to comparator (rabies) vaccine recipients;.
(2) Antibody recognition of bacterial proteins in whole cell preparations (direct binding to bacteria).
(3) IL-8 chemokine protection assay.

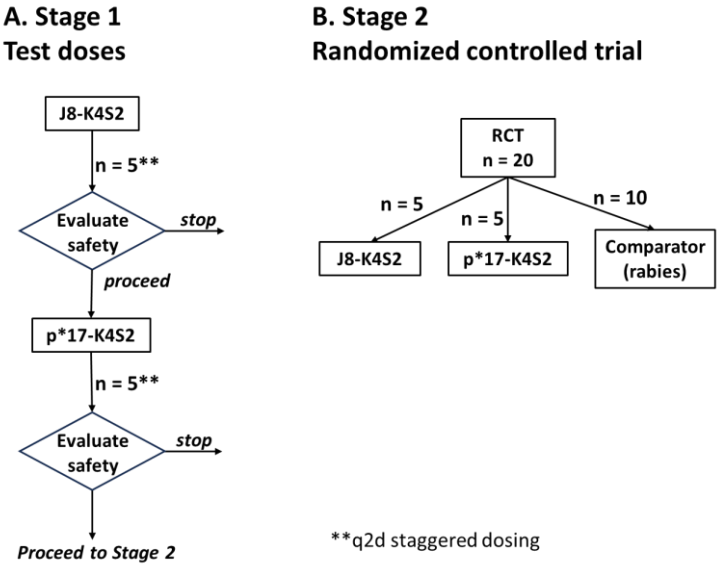
**for the purpose of this SAP, only the primary objectives are to be assessed first and independently of the secondary objectives as assay and efficacy data is ongoing.*

Section 3: Study Methods

3.1 Trial design

This single-site phase I, two-stage clinical trial in Edmonton, Alberta, Canada, recruited 30 healthy volunteers, aged 18–45 years, without any evidence of pre-existing valvular heart disease. The trial was divided into the initial unblinded safety test dose stage (stage 1) and the randomized, double-blinded, controlled trial stage (stage 2). Stage 1 recruited 10 volunteers—5 each to receive either J8-K4S2 or p*17-K4S2 in an unblinded, staggered fashion, whereby volunteers are dosed with intentional spacing of at least 2 days in between doses to monitor for any immediate side effects before dosing the next. Once all 5 volunteers had received 3 doses of the first test vaccine, a similar process followed for the second test vaccine. After

establishing initial safety in stage 1, we proceeded to stage 2, which recruited 20 volunteers to our 3-arm randomized controlled trial (RCT), to receive either of the trial vaccines, J8-K4S2 or p*17-K4S2, or comparator (rabies) vaccine, in a 1:1:2 ratio. All product doses occurred at 0, 3, and 6 weeks. The primary outcome is vaccine safety; the secondary outcome is immunogenicity and comparative analyses of the different vaccine regimens.



3.2 Randomization

Participants were randomized to receive either of the trial vaccines, J8-K4S2 or p*17-K4S2, or comparator (rabies) vaccine, in a 1:1:2 ratio. Randomization was performed by statistician, Maryna Yaskina, and code supplied to pharmacy for product distribution.

3.3 Sample size

Stage 1 recruited 10 volunteers—5 each to receive either J8-K4S2 or p*17-K4S2;
Stage 2 recruited 20 volunteers – 5 each to receive either J8-K4S2 or p*17-K4S2 and 10 to receive the comparator rabies vaccine.

3.4 Framework

Comparisons for the trial will be conducted under a descriptive statistics framework.

3.5 Timing of final analysis

The final analysis for will take place in one stage. The main report of the trial will be prepared after every patient has reached 1 week after their last follow-up visit to allow for final collection

of the AEs and investigations and all data has been collected, cleaned and the database has been "closed".

Section 4: Statistical Principles

4.1 Confidence intervals and P values

A two-sided level of 0.05 will be used to declare significance.

4.2 Adjustments for multiplicity

None

4.3 Confidence intervals to be reported

A 95% confidence interval will be reported around estimates of the treatment effects of the primary and secondary outcomes.

4.4 Adherence and protocol deviations

4.4.1 Adherence

Adherence will be determined by completion of all scheduled doses. There were a total of 3 doses of vaccine administered at Day 0, Day 21 +/- 7, and Day 42 +/- 7 for both stages.

4.4.1 Protocol deviations

Major protocol deviations are defined as:

1. Enrollment of ineligible patient
2. Failure to obtain informed consent or improper consent procedure
3. Patient randomized but receives none of the study interventions.
4. Incorrect bottle number taken by patient.

Protocol deviations are classified prior to unblinding of treatment. The number (and percentage) of patients with major and minor protocol deviations will be summarised by treatment group with details of type of deviation provided. The patients that are included in the ITT analysis data set will be used as the denominator to calculate the percentages. No formal statistical testing will be undertaken.

4.5 Analysis populations

All analyses will adhere to the principle of intention-to-treat. The ITT population will include all participants who were randomized in the trial. Additional analyses will be conducted on the per protocol and safety populations. The per protocol population will include all patients who were

adherent to the treatment, i.e. received all scheduled vaccination doses. The safety population will consist of all patients who took at least one dose of study drug. These additional analyses will only be presented if there is a substantial difference in these populations compared to the ITT population.

Section 5: Trial Population

5.1 Eligibility

We will use the CONSORT-2010 flow diagram to provide details of the number of patients screened followed by a breakdown of how many patients were eligible and how many were excluded due to violating our inclusion/exclusion criteria outlined in section 6.2 of the trial protocol.

5.2 Recruitment

A CONSORT-2010 flow diagram will be used to summarise the number of patients were assessed for eligibility at screening for both trials.

- eligible at screening
- ineligible at screening*
- eligible and randomised
- eligible but not randomised*
- received the randomised allocation
- did not receive the randomised allocation*
- lost to follow-up*
- discontinued the intervention*
- randomised and included in the primary analysis
- randomised and excluded from the primary analysis*

*reasons will be provided.

5.3 Withdrawal/follow-up

5.3.1 Timing of withdrawal/lost to follow-up

Timing of withdrawal and lost to follow up data will be presented in CONSORT diagram format with numbers and reasons for withdrawal and/or exclusion from analysis given at each stage.

5.3.2 Presentation of withdrawal data

The numbers (with reasons) of losses to follow-up (drop-outs and withdrawals) over the course of the trial will be summarised by treatment arm.

5.4 Baseline patient characteristics

Patients will be described with respect to age, sex, pre-existing health conditions, risk status, number of days from symptom onset to treatment initiation, provincial health zone, and race.

The demographic and clinical variables at baseline will be presented using the appropriate descriptive statistics, namely: means, standard deviations, medians and interquartile ranges for continuous variables, and frequency with proportions for discrete variables. These variables will be summarized by treatment group.

Section 6: Analysis

6.1 Outcome definitions List

Primary Safety Outcomes:

1. Clinical symptoms and signs
    - Clinical symptoms (physical assessment) | desc = descriptive text; cs = clinical significance
        - Date of physical exam: pe_date
        - General appearance:
            - general_appearance
            - appearance_desc
            - gen_appearance_cs
        - Head, Eyes, Ears, Nose & Throat (H.E.E.N.T):
            - heent
            - heent_desc
            - heent_cs
        - Neck/Thyroid:
            - neck
            - neck_desc
            - neck_cs
        - Respiratory:
            - respiratory
            - respiratory_desc
            - respiratory_cs
        - Cardiovascular:
            - cardio
            - cardio_desc
            - cardio_cs
        - Musculoskeletal:
            - muscle
            - muscle_desc

        - muscle_cs
    - Neurological:
        - neuro
        - neuro_desc
        - neuro_cs
    - Abdominal:
        - abdominal
        - abdominal_desc
        - abdominal_cs
    - Lymphatic:
        - lymphatic
        - lymphatic_desc
        - lymphatic_cs
    - Dermatologic:
        - dermatologic
        - derma_desc
        - dermatologic_cs
    - Other systems:
        - pe_other
        - pe_other1
        - pe_other1_norm
        - pe_other1_desc
        - pe_other1_cs
        - pe_other2
        - pe_other2_norm
        - pe_other2_desc
        - pe_other2_cs
- Vital signs (date of vital signs would be the same date of physical exam)
    - Weight: vs_weight
    - Height: vs_height
    - BMI calculated score: vs_bmi
    - Temperature: vs_temp
    - Systolic BP: vs_sbp
    - Diastolic BP: vs_dbp
    - Pulse: vs_pulse
    - Respiratory Rate: vs_resp

2. Standard laboratory parameters (hematology, biochemistry) | clinical significance completed if test assessment was abnormal
    - Pregnancy test (females)
        - Date of pregnancy test: preg_date
        - Type of pregnancy test: preg_type
        - Result of pregnancy test: preg_result
    - Laboratory test date: lab_dot

- Hematology:
    - RBC
        - Test result: lab_res6
        - Test assessment: lab_asses6
        - Clinical significance: lab_cs_6
        - Additional comments: lab_cmnt6
    - Hemoglobin
        - Test result: lab_res1
        - Test assessment: lab_asses1
        - Clinical significance: lab_cs
        - Additional comments: lab_cmnt1
    - Hematocrit
        - Test result: lab_res3
        - Test assessment: lab_asses3
        - Clinical significance: lab_cs_3
        - Additional comments: lab_cmnt3
    - Platelet
        - Test result: lab_res4
        - Test assessment: lab_asses4
        - Clinical significance: lab_cs_4
        - Additional comments: lab_cmnt4
    - Neutrophil
        - Test result: lab_res2
        - Test assessment: lab_asses2
        - Clinical significance: lab_cs_2
        - Additional comments: lab_cmnt2
    - Leukocytes/WBC
        - Test result: lab_res5
        - Test assessment: lab_asses5
        - Clinical significance: lab_cs_5
        - Additional comments: lab_cmnt5
- Lipid:
    - Triglycerides
        - Test result: lab_res8
        - Test assessment: lab_asses8
        - Clinical significance: lab_cs_8
        - Additional comments: lab_cmnt8
    - Cholesterol
        - Test result: lab_res11
        - Test assessment: lab_asses11
        - Clinical significance: lab_cs_11
        - Additional comments: lab_cmnt11
    - HDL Cholesterol

        - Test result: lab_res12
        - Test assessment: lab_asses12
        - Clinical significance: lab_cs_12
        - Additional comments: lab_cmnt12
    - LDL Cholesterol
        - Test result: lab_res13
        - Test assessment: lab_asses13
        - Clinical significance: lab_cs_13
        - Additional comments: lab_cmnt13
- Antibodies:
    - Anti-Streptolysin O antibodies
        - Test result: lab_res9
        - Test assessment: lab_asses9
        - Clinical significance: lab_cs_9
        - Additional comments: lab_cmnt9
    - Anti-DNAse B antibodies
        - Test result: lab_res10
        - Test assessment: lab_asses10
        - Clinical significance: lab_cs_10
        - Additional comments (typically includes date of test but not consistent, paper source available if actual dates are required): lab_cmnt10
- Chemistry:
    - ESR
        - Test result: lab_res_esr
        - Test assessment: lab_asses_esr
        - Clinical significance: lab_cs_esr
        - Additional comments: lab_cmt_esr
    - CRP
        - Test result: lab_res_crp
        - Test assessment: lab_asses_crp
        - Clinical significance: lab_cs_crp
        - Additional comments: lab_cmt_crp
    - Troponin
        - Test result: lab_res1_v2
        - Test assessment: lab_asses1_v2
        - Clinical significance: lab_cs1_v2
        - Additional comments: lab_cmnt1_v2
    - K^+
        - Test result: lab_res2_v2
        - Test assessment: lab_asses2_v2
        - Clinical significance: lab_cs2_v2
        - Additional comments: lab_cmnt2_v2

    - Glucose
        - Test result: lab_res3_v2
        - Test assessment: lab_asses3_v2
        - Clinical significance: lab_cs3_v2
        - Additional comments: lab_cmnt3_v2
    - Creatinine
        - Non-iSTAT (i.e., serum)
            - Test result: lab_res4_v2
            - Test assessment: lab_asses4_v2
            - Clinical significance: lab_cs4_v2
            - Additional comments: lab_cmnt4_v2
        - iSTAT (POC)
            - Test result: lab_res5_v2
            - Test assessment: lab_asses5_v2
            - Clinical significance: lab_cs5_v2
            - Additional comments: lab_cmnt5_v2
    - Na^+
        - Test result: lab_res_na
        - Test assessment: lab_asses_na
        - Clinical significance: lab_cs_na
        - Additional comments: lab_cmnt_na
    - Cl^-
        - Test result: lab_res_cl
        - Test assessment: lab_asses_cl
        - Clinical significance: lab_cs_cl
        - Additional comments: lab_cmnt_cl
    - CO_2
        - Test result: lab_res_co2
        - Test assessment: lab_asses_co2
        - Clinical significance: lab_cs_co2
        - Additional comments: lab_cmnt_co2
- Liver Enzymes
    - AST
        - Test result: lab_res6_v2
        - Test assessment: lab_asses6_v2
        - Clinical significance: lab_cs6_v2
        - Additional comments: lab_cmnt6_v2
    - ALT
        - Test result: lab_res7_v2
        - Test assessment: lab_asses7_v2
        - Clinical significance: lab_cs7_v2
        - Additional comments: lab_cmnt7_v2
- Urinalysis

    - Date of urinalysis: lab_dot_v3
    - Leukocyte
        - Test result: lab_res1_v3
        - Test assessment: lab_asses1_v3
        - Clinical significance: lab_cs1_v3
        - Comments: lab_cmnt1_v3
    - Protein
        - Test result: lab_res2_v3
        - Test assessment: lab_asses2_v3
        - Clinical significance: lab_cs2_v3
        - Comments: lab_cmnt2_v3
    - Blood
        - Test result: lab_res3_v3
        - Test assessment: lab_asses3_v3
        - Clinical significance: lab_cs3_v3
        - Comments: lab_cmnt3_v3
        - WBC (if blood was detected, microscopy)
            - Test result: lab_res4_v3
            - Test assessment: lab_asses4_v3
            - Clinical significance: lab_cs4_v3
            - Comments: lab_cmnt4_v3
        - RBC (if blood was detected, microscopy)
            - Test result: lab_res5_v3
            - Test assessment: lab_asses5_v3
            - Clinical significance: lab_cs5_v3
            - Comments: lab_cmnt5_v3
    - General comments: abnormal urinalysis results not asked may be captured here (i.e., specific gravity, bacteria, etc.)
        - gen_com
- Throat swab (culture)
    - Date of throat swab test: lab_date_cult_v3
    - Culture
        - Test result: lab_asses8_v3
        - Comments: lab_cmnt8_v3
    - Rapid antigen test
        - Test result: lab_asses9_v3
        - Comments: lab_cmnt9_v3
- ECG
    - Date of ECG: date_ecg
    - Test assessment: lab_asses10_v3
    - Clinical significance: lab_cs10_v3
    - Description (if ECG is clinically significant): lab_cmnt10_v3
    - Comments: ecggen_com

3. Echocardiogram findings (mitral regurgitation)
    - Date of ECHO: date_echo
    - Test assessment: lab_asses11_v3
    - Clinical significance: lab_cs11_v3
    - Description (if ECHO is clinically significant): lab_cmnt11_v3
    - Comments: echogen_com

Secondary Immunogenicity Outcomes*:

1. Antibody titers before and after 1, 2, and 3 doses of Strep A combination peptide vaccines and compare titers to comparator (rabies) vaccine recipients
2. Antibody recognition of bacterial proteins in whole cell preparations (direct binding to bacteria)
3. IL-8 chemokine protection assay

**To be assessed later when the data is completed. For the purpose of this SAP, only the primary objectives are to be assessed first and independently of the secondary objectives as assay and efficacy data is ongoing.*

Secondary Safety Outcomes:

1. Total number and number of subjects with AEs and SAEs
2. Total number and number of subjects with AEs and SAEs by toxicity grading
3. Local AEs (pain, redness, swelling at injection site)
4. Systemic AEs (fever, headache, myalgia, malaise)

6.2 Analysis methods

6.2.1 Analysis methods used

All primary and secondary analyses will be descriptive, total and relative frequencies (overall and by arm) will be given for categorical variables. Continuous variables will be summarized by mean, standard deviation, median, quartiles, maximum and minimum values.

6.2.3 Sensitivity analyses

None planned

6.3 Subgroups

Due to a very small sample size, no subgroup analysis is planned

6.4 Missing data

No missing data is expected. The analysis will be performed on the available data.

6.5 Statistical software

The statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R (R Core Team, Vienna, Austria) will be used for the analysis.