Trials

STUDY PROTOCOL

Open Access

A phase 1 randomized controlled trial of a peptide-based group A streptococcal vaccine in healthy volunteers

Vanessa Meier-Stephenson[1,2,3*†], Michael T. Hawkes[2,4†], Catherine Burton[4], Ainslie Calcutt[5], Chris Davis[5], Jessica Dooley[5], Michael Good[3,5], Michael Houghton[2,3], Eloise Keeffe[5], Kelly Kim[6], Ailin Lepletier[5], Conar O'Neil[1], Ijeoma Ogbuehi[6], Victoria Ozberk[5], Manisha Pandey[5], Simone Reynolds[5], Avi Seth[6], William Stokes[1,7], Alena Tse-Chang[4], Ben Tyrrell[9], D. Lorne Tyrrell[2,3], Gregory J. Tyrrell[7] and Maryna Yaskina[8]

Abstract

Background 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 (CRM_{197}), and the conjugated peptide vaccines are abbreviated as J8-K4S2 or p*17-K4S2.

Methods This single-site phase I, two-stage clinical trial in Edmonton, Alberta, Canada, aims to recruit a total of 30 healthy volunteers, aged 18–45 years, without any evidence of pre-existing valvular heart disease. The trial is divided into the initial unblinded safety test dose stage (stage 1) and the randomized, double-blinded, controlled trial stage (stage 2). Stage 1 will recruit 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 have received 3 doses of the first test vaccine, a similar process will follow for the second test vaccine. Once safety is established in stage 1, we will proceed to stage 2, which will recruit 20 volunteers to our 3-arm randomized controlled trial (RCT), receiving either of the trial vaccines, J8-K4S2 or p*17-K4S2, or comparator (rabies) vaccine. All product dosing will be 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.

Discussion This Strep A vaccine clinical trial aims to investigate safety and immunogenicity of two novel conjugated peptide-based vaccines, J8-KS42 and p*17-K4S2. If one or both vaccine products demonstrate favorable primary and secondary outcomes, the product(s) will move into phase II and III studies.

[†]Vanessa Meier-Stephenson and Michael T. Hawkes are co-first authors.

*Correspondence:
Vanessa Meier-Stephenson
meierste@ualberta.ca
Full list of author information is available at the end of the article

Trial registration ClinicalTrials.gov Identifier: NCT04882514. Registered on 2021–05-12, https://clinicaltrials.gov/study/NCT04882514.

Keywords Group A streptococcus (GAS; Strep a), Phase I vaccine clinical trial, M protein, Spy-CEP, *Streptococcus pyogenes*

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	A Phase 1 Randomized Controlled Trial of a Peptide-Based Group A Streptococcal Vaccine in Healthy Volunteers.
Trial registration {2a and 2b}.	ClinicalTrials.gov Identifier: NCT04882514.
Protocol version {3}	Protocol Identifying Number PRO 00089919; Version date: Oct 5, 2023.
Funding {4}	This is an Investigator-Initiated trial, sponsored by the University of Alberta and the Li Ka Shing Institute of Virology (Canada). The Trial and preliminary research is/are funded through the Canadian Institutes of Health Research (CIHR) Phase 1/2 Clinical Trials Grant program (Canada), the Heart Foundation (Australia); the Snow Foundation (Australia); the National Health and Medical Research Council of Australia; the National Foundation for Medical Research and Innovation (Australia); and the Lowitja Foundation (Australia).
Author details {5a}	[1]Department of Medicine/Division of Infectious Diseases, University of Alberta, Edmonton, AB, Canada [2]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB, Canada [3]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada [4]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada [5]Laboratory of Vaccines for the Developing World, Institute for Biomedicine and Glycomics, Griffith University, Queensland, Australia [6]Clinical Trials Office, University of Alberta, Edmonton, AB, Canada.
Name and contact information for the trial sponsor {5b}	D. Lorne Tyrrell, OC, AOE, MD, PhD, FRCPC, FRSC, FCAHS Professor and Director, Li Ka Shing Institute of Virology; Department of Medical Microbiology and Immunology, University of Alberta Edmonton, Alberta, Canada Phone: 1-(780)-492–8415 Email: lorne.tyrrell@ualberta.ca
Role of sponsor {5c}	The study sponsor, DLT via the Li Ka Shing Institute of Virology has aided in the study design, but has not played a role in data collection, management, analysis or interpretation of data. The study funder is a governmental funding agency (CIHR) and has evaluated the study protocol independently, but has no role in the operation, analysis or interpretation of the study data.

Introduction

Background and rationale {6a}

Group A streptococci (Strep A) or *Streptococcus pyogenes* causes an estimated 500,000 deaths worldwide each year [1]. Disease presentations vary from mild pharyngitis to skin infections with the most severe being necrotizing fasciitis, septicemia, and in rare cases streptococcal toxic shock. Even in some of the milder forms, untreated Strep A infection can lead to the serious post streptococcal pathologies of rheumatic fever/rheumatic heart disease and post-streptococcal glomerulonephritis [2]. These sequelae are thought to arise as the result of an autoimmune response against different Strep A proteins. There is a great need for a Strep A vaccine worldwide. This challenge, however, needs to be met with the balance of efficacy and a clear avoidance of autoimmunity.

A major virulence factor for Strep A is the M protein, a membrane-bound protein produced from the *emm* gene, where the outermost amino (N-) terminal segment defines the serotype. Each strain of Strep A expresses only one type of M protein, but there are greater than 250 serotypes. Unfortunately, there is limited cross-strain specific immunity adding to the challenge of vaccine development. There are also geographic variations in predominant circulating Strep A serotypes [3]. While some vaccine strategies have tried incorporating multiple M protein serotypes, there are inherent limitations to this strategy [4–7]. Other strategies consider alternate non-M protein targets [4, 8].

We have developed two Strep A vaccines based on a conserved minimal epitope from the surface M protein combined with an additional, non-M protein virulence factor—*S. pyogenes* cell envelope proteinase protein (SpyCEP). For the *M protein-based component*, two minimal epitope-based vaccines have been designed

against the conserved C3 repeat region—(1) the J8 peptide, which is a minimal 12-amino acid (aa) peptide flanked by amino acids from a yeast protein (GCN4) for structural stability, and (2) the p*17 peptide, which is a variant designed through aa substitutions [9–12]. Bioinformatics and in vitro analysis found no cross-reactivity to human heart proteins [13]. Both peptides were chemically conjugated to cross-reacting material (CRM_{197}) and adsorbed to aluminum hydroxide (Alum) for improved immunogenicity.

To further improve vaccine efficacy, a second epitope, based on the SpyCEP virulence factor, was also incorporated. This epitope has previously demonstrated modest efficacy against Strep A on its own [14]. J8-specific Abs require neutrophils for opsonic phagocytosis and J8-DT alone was ineffective against highly virulent Strep A strains that have upregulated SpyCEP [15, 16]. SpyCEP cleaves CXC chemokines, IL-8 (human), and KC and MIP-2 (mouse) responsible for attracting neutrophils to the site of infection. This lack of vaccine efficacy in the upregulated strains was reversible when combined with an inactive recombinant fragment of SpyCEP [16]. For the *SpyCEP component*, a 20-aa epitope was identified that did not have any IL-8 protease activity but has maintained the ability to induce neutralizing and protective antibodies [17]. The SpyCEP epitope is called K4S2 and was also conjugated to CRM_{197}. The combination of these two vaccines components above, create the combination vaccines: J8-K4S2 and p*17-K4S2.

We propose to undertake a phase 1 double-blind randomized controlled trial in healthy volunteers to test the safety and immunogenicity of these novel vaccines. The trial will have 3 arms: 2 experimental vaccine groups and 1 comparator vaccine group (Fig. 1).

The working hypothesis is that vaccination will be safe and will lead to antibodies that will be functional against multiple strains of Strep A in vitro.

Objectives {7}

1. Monitor and document possible adverse events occurring in healthy volunteers receiving Strep A vaccine, including cardiac valve assessment (echocardiogram).
2. Measure serum antibody titers before and after 1, 2, and 3 doses of Strep A combination peptide vaccines and compare titers to comparator (rabies) vaccine recipients.
3. Perform functional antibody assays (direct binding to bacteria, blockade of IL-8 proteolysis) using pre-immune and immune serum from the volunteers.

Trial design {8}

The study is divided into two stages—stage 1 is an open-label test dose stage for safety of the two vaccines (J8-K4S2 or p*17-K4S2)—ten (10) participants, and stage 2 is the double-blind RCT—twenty (20) participants, evaluating the two vaccines against a comparator rabies vaccine (RabAvert) (Fig. 1).

In order to maximize safety, the study will be conducted in two phases, with test doses administered to five

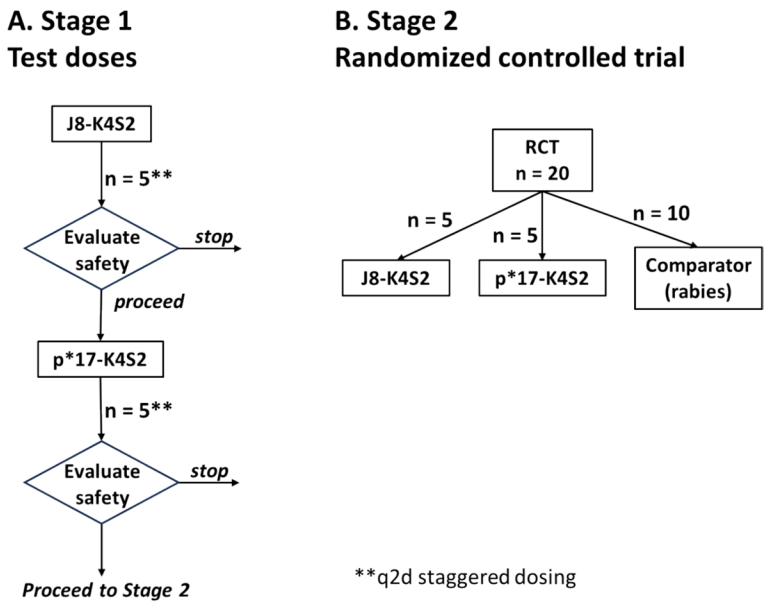

Fig. 1 Clinical trial flowchart of enrolment and interventions

participants for each product (J8-K4S2 and p*17-K4S2) (stage 1) and a fully randomized stage (stage 2). The trial profile is illustrated in Fig. 1, where the safety checkpoints for stage 1 are shown. Any severe adverse event (SAE), grade 4 AE, clinically significant valve abnormality on echocardiography, or episode of acute rheumatic fever (ARF) will require trial to be halted, at least temporarily, for DSMB review.

Following this stage 1 challenge, if no safety concerns arise, we will proceed to stage 2, with randomized allocation to each arm for 20 more participants who will receive J8-K4S2 vaccine ($n=5$), p*17-K4S2 vaccine ($n=5$), and 10 who receive comparator (rabies) vaccine.

The decision to proceed to stage 2 will occur as follows. Once all participants in stage 1 have received 3 doses of the J8-K4S2 or p*17-K4S2 vaccines (total 10 participants) and have completed visit 4 (2 weeks after the third vaccine dose), we will review safety data. Note that we will not require participants in stage 1 to complete visit 5 (6 months after the third vaccine dose) prior to proceeding to stage 2. The purpose of visit 5 is to assess longevity of the antibody response, and safety concerns are unlikely to arise between visits 4 (2 weeks after 3rd dose) and visit 5 (6 months after 3rd dose). The safety evaluation will be performed by the trial medical experts after stage 1 and will be reviewed by the Data Safety Monitoring Board (DSMB). The safety assessment will be based on standardized NIAID toxicity tables (Appendix 2) [18]. Any SAE or grade 4 AE will be used as a criterion for halting the trial for safety review.

Contingency plan in case of a safety concern in stage 1. If a safety signal occurs (i.e., SAE, grade 4 AE, new valve abnormality, or ARF) during stage 1, the DSMB will be involved, and a decision will be made about final dosing regimens for stage 2 (randomized stage). For example, if p*17-K4S2 (56.25 μg) is not tolerated, the trial may still proceed with J8-K4S2 and comparator (rabies) vaccine arms.

Methods: participants, interventions and outcomes

Study setting {9}

The study is a single-center, double-blinded randomized controlled trial taking place at the University of Alberta Hospital, Edmonton, AB, Canada. Healthy volunteers are recruited using local and online marketing tools to initiate pre-screening and collect contact information.

Eligibility criteria {10}

Inclusion criteria

1. Able to understand the purpose and the procedures involved in this study and sign the informed consent form.
2. Male or non-pregnant female adults, 18–45 years of age inclusive.
3. Non-smoker and in good general health, as determined by medical screening evaluation, performed by PI, or delegated sub-investigator no greater than 28 days before the first dose in the form of medical history, clinical laboratory tests, and physical examination.
4. Electrocardiogram (ECG) that is normal or with findings that are considered trivial and clinically insignificant or normal variations.
5. Echocardiogram (ECHO) that is normal or with findings that are considered trivial and clinically insignificant such as 'Clinically insignificant/trivial mitral regurgitation'.
6. Women must agree not to become pregnant during the trial. If they are sexually active, they must use an effective method of birth control, e.g., insertable, injectable, transdermal, or combination oral contraceptive approved by Health Canada combined with a barrier contraceptive and have negative results on a serum or urine pregnancy test done before administration of study medication.
7. Intention to reside in the geographical area for next 10 months and not intending to travel overseas for at least 30 days following the last study vaccine administration.
8. Agree not to participate in any other clinical trial during the trial.
9. Agree not to donate blood for the duration of the trial.
10. Agree to restrain from intensive physical exercise, i.e., exercise that varies significantly from an everyday exercise routine, 3 days before and after (±3 days) administration of each dose, including each interim visit for blood sample collection.
11. Up to date on seasonal influenza vaccine and recommended COVID-19 vaccines and booster doses at the time of study enrolment.

Exclusion criteria

1. Personal or family history of post-streptococcal disease (rheumatic fever or glomerulonephritis), or collagen-vascular disease.
2. Evidence of increased cardiovascular disease risk (defined as > 10%, 10-year risk using Framingham score—see Appendix 1). Risk factors include sex, age, systolic blood pressure (mm Hg), smoking status, body mass index (BMI, kg/m^2), reported diabetes status, and blood pressure.
3. Previous use of phentermine (appetite suppressant of the amphetamine and phenethylamine class),

fenfluramine, or dexfenfluramine known as Fen-Phen, anti-obesity medications (possible association with cardiac valvular abnormalities).

4. Clinical diagnosis or evidence of recent group A streptococcal infection as measured by anti-streptolysin O or anti-DNase B levels exceeding 200 units.
5. Positive group A streptococcus throat culture or rapid antigen test at screening.
6. Presence of significant acute infection requiring systemic antibiotic treatment within the 14 days prior to each product administration.
7. Pregnant or breast feeding (all women will have a negative pregnancy test result prior to each study product administered).
8. Immunized or intent to immunize with any vaccine or investigational agents within 30 days prior to enrolment through to 30 days following the last study vaccine administration, with the exception of licensed inactivated influenza vaccines and COVID-19 vaccines.
9. Past significant reaction following any previous vaccination.
10. History of hypersensitivity to any diphtheria toxoid or CRM_{197} containing vaccine.
11. Presence of acute infectious disease or fever (e.g., sub-lingual temperature 38.5 °C) within the 5 days prior to study product administration.
12. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immunodeficiencies), insulin dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, psoriasis, rheumatoid arthritis, asthma, epilepsy or obsessive–compulsive disorder, skin carcinoma excluding non-spreadable skin cancers such as basal cell and squamous cell carcinoma.
13. Evidence and any history of leukemia, lymphoma, or neoplasm.
14. Presence or suspicion of impaired immune system function. Currently receiving or having within the past 3 years received immunosuppressive therapy, including systemic steroids, adrenocorticotropic hormone (ACTH), or inhaled steroids in dosages that are associated with hypothalamic–pituitary–adrenal axis suppression, such as 1 mg/kg/day of prednisone or its equivalent or chronic use of inhaled high potency corticosteroids [budesonide 800 μg per day or fluticasone 750 μg].
15. Received blood, blood products, or a parenteral immunoglobulin preparation in the past 12 weeks.
16. Evidence of bleeding diathesis or any condition that may be associated with a prolonged bleeding time.
17. Known inherited genetic anomaly (known as cytogenic disorders), e.g., Down's syndrome.
18. Evidence of any condition that, in the opinion of the clinical investigator, might interfere with the evaluation of the study objectives or pose excessive risks to participants.
19. Findings of definite, probable, or possible rheumatic heart disease (RHD), definite or probable acute rheumatic fever (ARF).
20. Inadequate echocardiographic windows for assessment.
21. Echocardiographic findings such as cardiac chambers: left ventricular dilatation (based on LV diameter > 29 mm/m^2 to BSA); left ventricular dysfunction (ejection fraction < 50%; left ventricular hypertrophy (LV wall thickness > 11 mm); right ventricular dysfunction or dilatation (subjective assessment).
22. Cardiac valves/hemodynamic findings: clinically significant mitral regurgitation defined: at the discretion of the cardiologist and/or effective regurgitant orifice area of > 10 mm^2; any degree of valvular stenosis or left ventricular outflow tract obstruction; pulmonary hypertension (defined as an estimated right ventricular systolic pressure of > 30 mmHg, calculated using the peak tricuspid regurgitant jet velocity method).
23. Any aortic regurgitation.
24. Pericardium: greater than trivial pericardial fluid (trivial defined as < 5 mm and not circumferential).
25. Pre-existing significant structural valve disease (for example, but not limited to bicuspid aortic valve regardless of hemodynamic effect, mitral valve prolapse regardless of severity of regurgitation, pulmonary stenosis).
26. Other significant congenital lesions (for example, but not limited to, aortic coarctation, septal defect, excluding patent foramen ovale) (NOTE: findings considered normal developmental variation, specifically including patient foramen ovale and prominent Eustachian valve will not be considered exclusion criteria).
27. Clinical or sub-clinical acute post-streptococcal glomerulonephritis (APSGN).
28. Clinically significant abnormal laboratory results, e.g., CBC with differential and platelets, AST, ALT, creatinine, random blood sugar, electrolytes (including sodium, potassium, chloride, and bicarbonate); clinically significant proteinuria [in isolation of any other abnormalities] would be defined as 2 + (1.0 g/L) or 3 + (3.0 g/L) if not decreasing on

subsequent U/A or explained by other features. Trace proteinuria or 1 + (0.3 g/L) would be eligible as long as a repeat was normal before dosing start.

29. The participant has a diagnosis of schizophrenia, bi-polar disease, or other severe (disabling) chronic psychiatric diagnosis.
30. The participant has been hospitalized within the past 5 years prior to enrollment for psychiatric illness, history of suicide attempt, or confinement for danger to self or others.
31. The participant is receiving psychiatric drugs, but their psychiatric conditions are not stabilized. Participants who are receiving a single antidepressant drug and are stable for at least 3 months prior to enrollment without decompensating are allowed enrollment into the study; *aripiprazole, clozapine, ziprasidone, haloperidol, molindone, loxapine, thioridazine, thiothixene, pimozide, fluphenazine, risperidone, mesoridazine, quetiapine, trifluoperazine, trifluprom azine, chlorprothixene, chlorpromazine, perphenazine, olanzapine, carbamazepine, divalproex sodium, lithium carbonate, or lithium citrate. This is not an absolute contra-indication and clinician judgment will be used to assess the likelihood that this will compromise trial participation and follow-up.
32. The participant has a history of alcohol or drug abuse in the 5 years prior to enrollment. This is not an absolute contra-indication and clinician judgment will be used to assess the likelihood that this will compromise trial participation and follow-up.

Who will take informed consent? {26a}

Participants will be consented on-site by a dedicated research study coordinator. The informed consent form can be provided by the corresponding author upon request.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Written informed consent will be obtained from all trial participants, whereby the process shall be initiated at the time of enrolment into the study. The study will include an optional biobanking component which requires a separate consent signature if the participant agrees to participate. Samples will be collected at every study visit from enrolment onwards.

The informed consent form outlines the potential for use of the biobanked samples and study data by qualified researchers, only after appropriate Research Ethics Board approval, and could include researchers of the national and international research community (which may include researchers from academia, charitable organizations and "for-profit" private companies, such as drug companies).

The purpose of the optional biobanking study is to assess markers of innate and adaptive immune response to the vaccine.

Interventions

Explanation for the choice of comparators {6b}

The comparator vaccine, rabies vaccine, was chosen for its condensed dosing schedule and the fact that most study volunteers would not have previously received this vaccine before and could therefore act as a reasonable comparator for any post-vaccination reactogenicity. In addition, this would mean the volunteers of the comparator arm would also gain potential personal benefit from the study injections.

Intervention description {11a}

Stage 1: Open-label safety test doses: For each of the two investigational products, five (5) participants will receive a 3-dose series of one of the products.

Dose: This stage will include the dosing of the two products as intended for the stage 2 (RCT) component.

1. J8-CRM_{197} (50 μg) + K4S2-CRM_{197} (6.25 μg): total 56.25 μg
2. p*17-CRM_{197} (25 μg) + K4S2-CRM_{197} (6.25 μg): total 31.25 μg

Dosing schedule: 0, 3, and 6 weeks (3 doses). Dosing will occur in a staggered fashion to ensure optimal safety monitoring. Beginning with J8-K4S2, the first participant will be dosed and monitored for at least 2 days before dosing the second participant, who will similarly be monitored for at least 2 days before dosing the third and so on, until 5 participants have received their first dose of the product. Next, after 3 weeks, we will proceed with second dose of the vaccine product, beginning with the first volunteer, waiting 2 days, then dosing the second volunteer and so on until all have received their second dose. Finally at 6 weeks, we will dose with the third dose, staggering to catch any concerning safety signal. Dosing of the p*17-K4S2 will follow a similar staggered dosing pattern, ensuring close monitoring for any safety concerns among participants.

Route: Intramuscular. The injections should alternate between the left and right deltoid for each injection, but the participant can still opt for either arm at each visit.

Stage 2: Double-blinded RCT: Twenty (20) participants will be randomized to receive either one of the

investigation products or the comparator (rabies) vaccine (RabAvert).

Dose: This study will use J8-K4S2, p*17- K4S2, or a comparator (rabies) vaccine arm:

1. J8-CRM_{197} (50 μg) + K4S2-CRM_{197} (6.25 μg): total 56.25 μg
2. p*17-CRM_{197} (25 μg) + K4S2-CRM_{197} (6.25 μg): total 31.25 μg
3. Comparator (rabies) vaccine (RabAvert)

Dosing schedule: 0, 3, and 6 weeks (3 doses).
Route: Intramuscular—as above.

Criteria for discontinuing or modifying allocated interventions {11b}

(a) When and how to withdraw subjects from the trial

If a participant experiences any serious adverse event (SAE) or grade 4 adverse event (AE) thought to be causally related to the vaccine, further doses of the vaccine will be held, and the Data Safety and Monitoring Board (DSMB) chairperson will be notified (see Standardized Toxicity Tables, Appendix 2) [18]. After a review of the case, further vaccines may be discontinued or may proceed if the SAE is judged not to be related to the vaccine. Vaccination may also be withheld at the discretion of the trial physician or the participant themselves. In all cases, attempts will be made to retain the participant in the trial for data collection and blood sampling according to protocol. The participant may also withdraw voluntarily from the study at any time.

(b) The type and timing of data to be collected for withdrawn participant

If for any reason a participant does not complete the study, the reason will be entered on the case report file (CRF). All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. The reason for the early termination will be recorded. The time to resolution of the abnormal result, and any clinical sequelae will also be recorded.

In case vaccine is discontinued for safety reasons, participants may be willing to continue to have data collected for primary and secondary outcomes, including bloodwork. Their results will be included in an intention-to-treat analysis.

(c) Whether and how participants are to be replaced

We will attempt to collect all data and bloodwork from participants enrolled in the study, even if the vaccine series is interrupted or discontinued. If the vaccine series is interrupted or discontinued for any reason, an additional patient may be enrolled to replace the patient.

If the reason for interruption or discontinuation of the vaccine series is voluntary patient withdrawal, or a protocol deviation or violation unrelated to the safety of the vaccine product, the patient will be immediately replaced without consultation with the DSMB; if the reason for interruption or discontinuation of the vaccine series is related to a safety concern, the decision to replace the patient will be carefully considered, in consultation with the DSMB.

Of note, replacement of participants who do not complete the vaccine series is necessary to proceed through stage 1 of the trial and on to stage 2 of the trial (Fig. 1). This is because safety checkpoints are built into the trial design, requiring five patients to complete dose 1 of the vaccine before proceeding to dose 2, five patients to complete dose 2 of the vaccine before proceeding to dose 3, and five patients to complete 3 doses of each vaccine product (stage 1) before proceeding to stage 2 of the trial. The follow-up for participants withdrawn from trial treatment will be conducted per protocol-specified visits if the participant is agreeable.

If vaccine series is stopped because of an adverse event or withdrawal of consent for any reason, the subject will be followed and treated until the abnormal parameter or symptom has resolved or stabilized. The adverse events will be followed to resolution and the follow-up evaluations recorded when the subject has stabilized.

Strategies to improve adherence to interventions {11c}

Vaccine administration will be performed by study staff and directly observed; therefore, adherence is assured as long as the participant attends the trial visit. Attempts will be made to contact trial participants ahead of their trial visits as a reminder and in follow-up in case of missed visits. Participants must remain at the study clinic for a minimum of 30 min following immunizations to monitor for any immediate adverse reactions. Participants are given a memory aide/journal at each dosing visit to keep track of any symptoms or side effects they experience during the 1 week following the immunizations (Memory Aide template available upon request). Additionally, the study coordinator calls the participant 2 and 8 (± 1) days after each dose to check in about any potential side effects or concerns.

Relevant concomitant care permitted or prohibited during the trial {11d}

The trial will enroll healthy participants. We will record any medications the patient is taking during the trial for unrelated conditions. Our inclusion criteria incorporate agreement to not be involved in other clinical trials during the enrollment of this trial.

Other vaccines during the trial period

Given the ongoing COVID-19 infections and annual influenza activity during winter months, participants may wish to receive vaccines to prevent these infections during their participation in the trial. A potential challenge may arise if a participant develops an adverse reaction to one of these vaccines during the trial period. For example, mRNA vaccines against COVID-19 are associated with rare cardiac adverse events (e.g., pericarditis and myocarditis). Such an adverse event could be (incorrectly) ascribed to the experimental Strep A vaccine. To address this challenge, we have as an inclusion criterion that participants should be up to date with the most current influenza and COVID-19 vaccines (including recommended booster doses). This will reduce the likelihood that participants will need to be vaccinated during the trial period. We do not propose to restrict participants from receiving other vaccines during the trial; therefore, this inclusion criterion is intended to reduce the probability that a participant would need one of these vaccines. One of the trial exclusion criteria is "Immunized or intent to immunize with any vaccine or investigational agents within 30 days prior to enrolment through to 30 days following the last study vaccine administration, with the exception of licensed inactivated influenza vaccines and COVID-19 vaccines." Of note, the influenza and COVID-19 vaccines are exceptions to this exclusion criterion, such that the trial is permissive for vaccination against influenza or COVID-19, although we are taking measures to reduce the probability that this would be necessary.

Provisions for post-trial care {30}

Participants will be in the study for a minimum duration of 10 months. Passive follow-up (if participant initiates contact with the study team) will continue thereafter to the end of the trial (i.e., the last visit of the last participant) to document any additional outcomes.

Outcomes {12}

Primary outcomes: safety

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

Secondary outcomes: immunogenicity.

1. Antibody titers.
2. Antibody recognition of bacterial proteins in whole cell preparations (direct binding to bacteria).
3. IL-8 chemokine protection assay.

Primary outcome

Adverse events will be documented using standard toxicity tables, adapted from the National Institute for Allergy and Infectious Diseases (NIAID) Division of Microbiology and Infectious Diseases (DMID) toxicity tables (Appendix 2) [18]. Clinical, laboratory, and ancillary data which will be collected for safety monitoring are shown in Appendix 2: Table 1.

Secondary outcomes

1. *Antibody titers*. Samples of sera and saliva will be collected from the participants prior to each injection and at the scheduled clinic visits as indicated (Table 1). Sera will be stored, and assays will be conducted on batched samples after all sera have been collected. Standard ELISA will be performed to determine the level of J8, p*17, K4S2, and CRM_{197}peptide-specific IgG levels in sera and saliva of all participants at the described time intervals. The relationship between the titer of vaccine-specific antibodies and the number of doses administered will be investigated. In addition, titers will be compared to comparator (rabies) vaccine recipients.
2. *Direct binding activity of antibodies* in vitro. The direct binding activity of the vaccine-induced antibodies will be determined for reference strains of Strep A or clinical isolates. In this assay, the direct binding of antibodies to Strep A proteins in whole cell preparations will be determined by ELISA and/or flow cytometry [13, 19].
3. *Chemokine protection assay*. The ability of vaccine-induced K4S2 antibodies to block cytokine proteolysis will be determined. For IL-8 protection assay, Strep A strains will be grown to stationary phase. The cell-free Strep A culture supernatants will be co-incubated with recombinant chemokines (IL-8 and serum from vaccinated and control human donors). The chemokine with media alone will be used as a positive control. Uncleaved chemokines will be

Table 1 Trial procedures and participant timeline for stage 1 and stage 2

	Screening	Enrolment	Follow-up visits				Unscheduled visit[e]
Study visit #	0	1	2	3	4	5	
Day	−28 to −7	0	21 ± 7	42 ± 7	56 ± 7	224 ± 7	As required
Clinical visit	X	X	X	X	X	X	X
Assessment of eligibility criteria	X						
Informed consent	X						
Randomization		X*					
Vaccine administered		X	X	X			
Baseline symptoms prior to injection and 30-min observation following vaccination		X	X	X			
Acute complaints/adverse events/serious adverse events		X	X	X	X	X	X
Vaccine specific antibodies		X	X	X	X	X	
Saliva for mucosal antibodies		X	X	X	X	X	
Review of concomitant medications	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X
Physical examination including cardiac auscultation	X	X	X	X	X	X	X
ASOT and anti-DNase B antibody levels	X	X	X	X	X	X	X
Throat swab for culture and rapid antigen test	X	X	X	X	X	X	X
CBC and differential, AST, ALT, glucose, electrolytes, ESR, CRP	X	X	X	X	X	X	X
Total cholesterol, HDL	X						
Creatinine[c]	X	X[c]	X[c]	X[c]	X	X	X
Troponin	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X
ECG	X	X	X	X	X	X	X
Pregnancy test (urine)	X[a]	X	X	X	X	X	X
Echocardiogram[d]	X	X	X	X	X	X	X
Memory aid		X	X	X			
Phone visit[b]		X	X	X			
Optional biobanking		X	X	X	X	X	

* Stage 2 only

[a] Blood serum pregnancy test will be performed at the screening visit

[b] The study team will call the participant 2 and 8 days after each injection to review the memory aid card and any potential side effects

[c] 3Point-of-care creatinine will be obtained prior to dosing

[d] Operational memo to allow Echo scheduling window to be ± 14 days

[e] Unscheduled visits with assessments performed as necessary

measured using commercial ELISA and neutralization of chemokine cleaving activity due to vaccine antiserum will be calculated in comparison to the controls (chemokine with normal serum/no serum or in media alone) [19].

Participant timeline {13}

The participant timeline is shown in Table 1.

Sample size {14}

Thirty (30) participants are to be enrolled—10 in J8-K4S2 arm, 10 in p*17-K4S2 arm, and 10 in comparator vaccine arm. This includes 10 patients in stage 1, for initial test doses, and 20 patients in stage 2, in a fully randomized, controlled stage (Fig. 1).

To calculate this sample size, we took the J8-specific antibody titer as the continuous immunogenicity design endpoint and used data from a pilot study of the J8-DT vaccine in 8 healthy volunteers [20]. The mean (SD) titer of antibody to J8 was 1.6 (0.29) μg/mL and 4.5 (2.4) μg/mL at baseline and 28 days after vaccination, respectively. Assuming the immunogenicity of the J8-K4S2 or p*17-K4S2 vaccine will be at least as strong, and using a t-test with $\alpha = 0.05$ and power = 0.8, eight subjects per group would be needed to demonstrate an increase in titers after vaccination compared to commercial vaccine

(RabAvert, by standard sample size calculations for normally distributed data.

Recruitment {15}

A professionally designed participant recruitment campaign (Marketing Martian (https://marketingmartian.ca/, Edmonton, AB) designed attractive Google, Facebook, and Instagram ads to recruit healthy volunteers. A prescreening questionnaire will allow potential participants to self-assess their eligibility; then, if wishing to take part, they can provide their contact information for the trial study coordinator to reach out to them. The campaign also alludes to compensation without elaborating on amounts.

Assignment of interventions: allocation

Sequence generation {16a}

For stage 2 of the trial, randomization will be in a ratio of 5:5:10 (J8-K4S2 vaccine: p*17-K4S2 vaccine: comparator (rabies) vaccine). The study statistician will create a randomization code list which will be forwarded to the research pharmacy.

Concealment mechanism {16b}

The pharmacy remains unblinded to the study products and, using the code generated by the statistician, prepares and sends the product to the study team in a concealed bag. Both the vaccine products and the RabAvert vaccine require reconstitution before administering, but because RabAvert has a different color than the study products, an unblinded nurse through the Clinical Investigation Unit will reconstitute the vaccine and administer as appropriate whereby any study team members on the unit will remain out of the room to maintain the blind.

Implementation {16c}

The randomization code generated by the statistician is given to pharmacy who assigns the intervention in sequence based on the provided code. Once a participant is deemed fully eligible, they are booked for their first dosing visit and given an enrolment study number, linking them to a specific vial allocation number.

Assignment of interventions: blinding

Who will be blinded {17a}

The stage 1 of the trial is unblinded, open-label; the stage 2 of the trial, the placebo-controlled RCT, is blinded. The randomization code for stage 2 will remain blinded to the study team (study coordinators, study monitors, trial physicians) and participants. For the purposes of administration, the study team will leave the room during reconstitution and administration of the product by a non-trial nurse. In addition, the laboratory team/basic scientists analyzing sample immunity and responses are also blinded to the allocation.

Procedure for unblinding if needed {17b}

The randomization code list for emergency unblinding purposes will be kept secure on site and is available through the pharmacy. If there is any safety concern (see above), all further dosing for that participant will stop and the DSMB will be notified immediately, where unblinding may likely be recommended.

Data collection and management

Plans for assessment and collection of outcomes {18a}

The initial data is collected and documented on individual data collection worksheets (screening form, enrolment case report file (CRF), clinic visit case report file; sample documents available upon request), then subsequently on the electronic medical record (Connect Care) as part of the participants' medical record. The study data is also input and tracked in Research Electronic Data Capture (REDCap) database, which is also employed to capture and track adverse events. REDCap also facilitates the data pull and analysis.

Screening form

Participants recruited to the trial will have a screening form completed, according to inclusion and exclusion criteria, listed above.

Enrolment case report file (CRF)

Eligible, consenting participants will undergo a full history and physical examination, the results of which will be recorded on the CRF. The following systems will be examined: vital signs, anthropometric data (height, weight), general appearance, ear, nose, and throat, cardiovascular, respiratory, musculoskeletal, gastrointestinal, dermatological, neurological. Demographic information, past medical history, immunizations, medications, and social history will be taken. Study entry blood work and assessments are done according to Table 1.

Clinical visits

At each visit, participants will be assessed for any change in their medical status, including intercurrent illness, new medications or vaccinations, and travel. A set of vitals and a focused physical assessment will be performed. Bloodwork for safety monitoring will be obtained at selected visits (Table 1). Urinalysis, throat swabs, echocardiograms, and an ECG will also be performed at the clinical visits. Serum and saliva will be

stored for assays for antibody levels (tested in batch at the end of the study).

Participants will be asked to record their temperature and symptoms on the day of injections and for 7 days following each injection on a memory aid card. The study team will perform a phone visit 2 and 8 days after each injection to review the memory aid with the participant.

Laboratory testing

Laboratory testing employs the standard diagnostic assays used by the Alberta Precision Labs Public Health laboratory. The exceptions to processing at this site are the bedside creatinine test—iSTAT Crea (Abbott), the ID NOW Strep A assay (Abbott), and the anti-DNase B test (Beckman Coulter, performed at British Columbia Centre for Disease Control Public Health Laboratory).

Training logs

Training of individuals with regard to the protocol and study-specific aspects are documented in the training log, and any study tasks are allocated according to team member role and qualifications and documented in the delegation log.

Plans to promote participant retention and complete follow-up {18b}

The study coordinator will contact participants for appointment bookings and follow-up visits, as well as for review of their memory aides post-dosing, as described above. For participants who discontinue or deviate from the intervention protocols, the study coordinator/study team member will contact them for ongoing follow-up assessments and investigations at the usual timepoints, even if not receiving any further dosing. This will be the case whether the withdrawal was for voluntary purposes or from a grade 4 AE, SAE, or other clinical criteria deemed by the study team as potential concern for continuing with dosing. Each visit is compensated for financially and proportionate to the time required.

Data management {19}

Data management responsibilities

The study coordinator will complete the screening form and document baseline health status on the enrolment CRF. They will also complete study forms at each visit detailing the clinical data of the patients (safety parameters). Physical assessment will be performed by the PI or sub-investigators. These forms will be collected and will form the clinical data for each study participant's folder. A folder will be created as soon as a patient is enrolled into the study. These folders will be kept in the data room in a locked cabinet. At admission, the medical history and physical examination forms will be completed. All data collection will be performed according to the SOPs for each type of testing, which will contain complete information for filling out each form, data entry, cross-checking of data entry, data cleaning, storage, and back-up.

Data will be deposited and maintained in a REDCap database which is hosted at the Women and Children's Health Research Institute (WCHRI) in Edmonton, Alberta, and reviewed in a blinded fashion by the team statistician. The database will be locked and patient data unblinded at the end of the cross over randomized controlled trial (i.e., 6 months after the last patient commences treatment or comparator vaccine).

Source documentation will be maintained by study personnel so that the conduct of the trial and treatment of study participants can be verified by monitoring oversight.

The sponsor and/or assigned designee will be responsible for the processing and quality control of the data. Source data including source documents, CRFs, copies of protocols and protocol amendments, drug accountability, correspondence, study logs, consent forms, and other essential documents for the study will be retained for at least 15 years after the termination/completion of the study.

No study document or image should be destroyed without prior written agreement between the sponsor and the investigator. Document destruction is subject to local regulations of retention. Should the investigator wish to assign the study records to another party or move them to another location, advance written notice should be given to the sponsor.

Data storage and back-up

Hard copies of forms will be kept in the study data room as described above. Weekly back-up of database files to a back-up drive will be performed as data is entered.

Timing/reports

Data review will be an ongoing process. Reports will be produced every 6 months unless there is need to report an unexpected result or problem after the findings has been confirmed and analyzed.

Safety monitoring will be ongoing and continuous, to allow identification of potential safety concerns.

Results will be compiled at the end of the study, after participant recruitment and follow-up is complete. Final publications will be based on the originally planned data analysis, or on appropriate modifications of this plan, if it

becomes clear that modifications or changes are necessary to best examine the data.

Study record retention

Paper copies of records will be maintained until the end of the study and for at least 15 years after that point, so that data can be cross-checked and verified with hard copies if necessary. After 10 years, if electronic records are determined to be accurate, hard copies will be destroyed. Electronic records will be maintained on password-protected computers and storage drives for 10 years. No special permission is required prior to destruction of records.

Confidentiality {27}

Study participant information will be kept strictly confidential. Study data will be accessible only to study personnel. At the point of data entry onto case report forms, and in the study database, information will contain only a unique study ID number and no other patient identifiers. A separate list, linking the study ID number to the participant name, will be kept separately in case there is a need to return to the medical record or source documents for any reason. This list, linking study ID to participant identifiers, will be kept strictly confidential in a separate password-protected file on a password-protected desktop computer in a locked office accessible only to study personnel. Study data will be stored long-term in files and computer databases in locked offices. Access to databases will be password-restricted, and network security measures will be in place to ensure that information cannot be retrieved by personnel not involved with the study.

Study participant confidentiality is strictly held in trust by the participating investigators, their staff and the sponsors and their agents. This confidentiality includes testing of biological samples in addition to clinical information.

No information concerning the study or the data will be released to any unauthorized third party without prior approval of the sponsor. The study monitor or other authorized representatives may inspect all documents and records required to be maintained by the PI, and the study site will permit access to such records.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

The study site coordinator will manage the study sample handling and storage in accordance with ICH guidelines and approved by the PI. The study site coordinator will review sample handling, storage, location, and shipping with the PI monthly. Discrepancies will be noted, recorded, discussed, and resolved with the PI.

A manual of SOPs will be developed to specify how all the laboratory samples shall be collected, transported, tested, and reported.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Primary outcomes

Safety data will be prospectively collected on standardized forms, by a trial nurse blinded to study arm. Standardized toxicity tables, adapted from the NIAID toxicity tables, are presented in Appendix 2 [18]. Frequencies of adverse events in each study arm will be tabulated (descriptive statistics). We do not propose to compute comparative statistics between study arms because of small patient numbers in each study arm.

Secondary outcomes

Antibody titers will be compared between experimental arms (J8-K4S2 and p*17-K4S2) and comparator (rabies) vaccine using standard statistical methods (e.g., Kruskal–Wallis test). Direct binding activity of antibodies in vitro will be captured through ELISA and/or flow cytometry and values compared per previous [13, 19]. Chemokine protection assay will be performed using a commercial ELISA and employ 2-way ANOVA for comparison of the groups.

Interim analyses {21b}

An interim analysis for safety and trial quality indices is planned after stage 1 (test doses). Data on the primary endpoint (safety) will be presented to the Data Safety and Monitoring Board (DSMB) for review, who may advise that the trial continue without modification, continue with changes to the protocol, or be discontinued. The DSMB will review any SAEs, grade 4 adverse events, or safety concerns on echocardiogram during stage 1 and stage 2 of the trial. During stage 2, the DSMB may request unblinding of the data to decide if AEs are vaccine related. With respect to the interpretation of safety data, the DSMB may recommend termination or modification of the trial if rates of adverse events appear disproportionately elevated. We do not propose to be guided by statistical thresholds given the small number of patients in each arm. Furthermore, we do not propose that the DSMB be strictly bound by pre-specified criteria, because of the complexity of the trade-offs between safety, efficacy, and the possibility that new information will change considerations. Rather, consideration of

stopping guidelines requires a reasoned judgment based on all information that is available at the time of data review.

Methods for additional analyses (e.g., subgroup analyses) {20b}

Given the small study numbers, no subgroup analyses are planned.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

If a participant is lost to follow-up or withdraws for any non-safety-related reason or is deemed no longer eligible or appropriate to continue in the study from the study team/clinical team perspective, that participant will be replaced in the study to ensure sufficient numbers for data analysis.

Plans to give access to the full protocol, participant-level data and statistical code {31c}

The protocol will be readily available; however, corresponding data generated from this trial will not be for the public. The trial has been registered with ClinicalTrials.gov under the identifier: NCT04882514, since 12 May 2021.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

Site initiation, ongoing eligibility review/monitoring, and study close out visits will be performed by the Northern Alberta Clinical Trials Centre (NACTRC), Clinical Trials Office within the University of Alberta (http://www.qmcr.ualberta.ca/). After stage 1, the study coordinators will provide the study statistician with all the safety data, who will compile a report of the findings and compilation of the AEs and SAEs found from stage 1. The Data Safety and Monitoring Board (DSMB), comprising of three members experienced in clinical trials and unassociated with the current trial, will meet for review of the stage 1 data and safety assessment prior to the initiation of stage 2. This post-stage 1 DSMB review meeting will be a closed meeting. A DSMB report will follow this along with recommendations to one of the following: (i) cease the trial, (ii) continue the trial as planned, or (iii) continue the trial with specific modifications. Data capture will be through the REDCap database, hosted by WCHRI at the University of Alberta and managed by the study coordinator. No additional stakeholders or public involvement groups are currently involved with this study.

Composition of the data monitoring committee, its role and reporting structure {21a}

The principal investigators are responsible for the supervision of data entry accuracy and adherence to protocol guidelines. They are also responsible for making sure that all study individuals complete appropriate training in research ethics and maintain up to date training in this area. In addition, any concerns about violations of ethical standards will be brought to the site PI, who will record the specific allegation, investigate the allegation, and discuss the findings with the PIs. The PIs will complete the investigation, address the concern, and record the investigation and outcome in study records.

Monitors will conduct interim monitoring visits to ensure compliance with Good Clinical Practices (GCP) and the study is conducted according to site-specific standard operating procedures (SOPs), the protocol, and regulatory guidelines. The main responsibilities of the monitor are to visit the investigator before, during, and after the study to ensure adherence to the protocol, all data are correctly and completely recorded and reported, and informed consents are obtained and recorded for all subjects before their participation in the study. The study monitor will contact and visit the investigator at regular intervals throughout the study. The monitor will be allowed to check and verify the various records (CRFs and other pertinent source data records) relating to the study to verify adherence to the protocol and to ensure the completeness, consistency, and accuracy of the data being recorded.

As part of the supervision of the study progress, other sponsor personnel may, on request, accompany the study monitor on visits to the study center. The investigator and assisting staff must agree to cooperate with the study monitor to resolve any problems, errors, or possible misunderstandings concerning the findings detected in the course of these monitoring visits.

Adverse event reporting and harms {22}

In this trial, adverse events will be systematically assessed and graded at each study visit and throughout the follow-up period (total 9 months) using standardized toxicity tables (Appendix 2) [18]. The data, initially recorded on paper for the study file, will be entered into an electronic database (REDCap). Frequencies of adverse events in each study arm will be reported (descriptive statistics). Comparative statistics will not be computed because of small patient numbers in each study arm.

As noted above, despite requiring up to date vaccination against influenza and COVID-19 at the time of trial enrolment (including criterion), participants may wish to be vaccinated against these infections during the study period (e.g., if a new vaccine becomes available during

the trial). Adverse events following administration of another vaccine (e.g., influenza or COVID-19) during the trial may need to be carefully considered. The DSMB will provide an arms-length judgment of the expectedness and relatedness of the adverse effect to the experimental vaccine and/or the co-administered vaccine. Adverse events judged to be attributable to the co-administered vaccine may not require trial halting or withholding future experimental vaccine doses, depending on the judgment of the DSMB.

For all SAEs, a report will be generated and made available to the DSMB chairperson. The University of Alberta HREB will be notified if the SAE is unexpected, related, or possibly related to the study intervention and suggests an increase in risk to study participants.

The investigator will follow-up on all adverse events and serious adverse events if not resolved at the initial report. If a participant has an SAE that is not resolved when the participant completes participation in the study, they will be followed until either:

- The event resolves
- The condition stabilizes
- The event returns to baseline
- The participant dies
- The event can be attributed to causes other than the study drug or procedures from the study

All serious unexpected adverse drug reactions will be reported to Health Canada and the local ethics board at the University of Alberta. The adverse events will be followed to resolution and the follow-up evaluations noted in adverse event CRF and recorded when the participant has stabilized.

Frequency and plans for auditing trial conduct {23}

On-site monitoring will be conducted regularly by internal monitors through the Northern Alberta Clinic Trials Centre (NACTRC), University of Alberta. The investigators and institution (University of Alberta) will facilitate all trial-related monitoring, audits by institutional review boards, and regulatory inspections by providing direct access to source data and documents. The on-site monitors will review the study data minimally every 2–3 weeks and provide reports to the study PI and study coordinator for review.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

All protocol amendments are reported to the REB for revision and approval. Any minor operational or technical amendments are implemented after approval; any operational amendments incorporating a change in safety criteria (i.e., inclusion/ exclusion criteria or monitoring) would be reported to both the REB and DSMB and, if necessary, to Health Canada for re-approval. If any amendments to participant involvement in the study (i.e., extra visits, extra bloodwork, varied compensation, etc.), the participant would be notified and re-consented if the requests fell outside of what was included in the initial informed consent form.

Dissemination plans {31a}

Following completion of the study, the investigators will communicate results of this research through publication in Open Access scientific journal(s). They will also be presenting the work at various research conferences and reporting results through the ClinicalTrials.gov website under the registered identifier: NCT04882514.

Discussion

As the first stage of the study (test doses) proceeded, the staggered dosing allowed for close monitoring and AE reporting of each participant before initiating a subsequent dose in another volunteer. The timing for this, given the assessment and test coordination, was such that as soon as all 5 participants were dosed with their first, the second dosing round started shortly thereafter with little room for delays or incidentals. In addition, the intent to show safety in 5 volunteers with the first (and subsequent) doses meant that if anyone from that stage were to leave [for any reason], goals would not be able to be met without first replacing that participant in a possible "catch-up period." Depending on the timing, this could lead to shifting all other participants' dosing out-of-window. Our team was ultimately able to coordinate this, but the stage's design held the potential to lead to gross protocol deviations, especially given the short dosing intervals of 0, 3, and 6 weeks placing additional time pressures on replacements. A revised contingency plan should be considered for future similar designs.

Trial status

Protocol version 05Oct2023. Recruiting for stage 2.

Initial recruitment start date: 07 November 2022; estimated date of completion: 31 March 2025.

Abbreviations

aa	Amino acid
ACTH	Adrenocorticotropic hormone
AE	Adverse event
ALT	Alanine transaminase
ANOVA	Analysis of variance
APSGN	Acute post-streptococcal glomerulonephritis
ARF	Acute rheumatic fever
ASOT	Antistreptolysin O titer
AST	Aspartate transaminase
COVID-19	Coronavirus disease of 2019

CRF	Case report form
CRM_{197}	Cross-reacting material 197
CRP	C-reactive protein
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
Echo	Echocardiogram
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
GAS	Group A streptococcus
HDL	High-density lipoprotein
IL-8	Interleukin-8
LV	Left ventricle
MVP	Mitral valve prolapse
NACTRC	Northern Alberta Clinical Trials Research Centre
PCR	Polymerase chain reaction
RCT	Randomized controlled trial
REDCap	Research Electronic Data Capture database
RHD	Rheumatic heart disease
RV	Right ventricle
SAE	Serious adverse event
SpyCEP	*Streptococcus pyogenes* Cell envelope proteinase
Strep A	Group A streptococcus
WCHRI	Women and Children's Health Research Institute

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08634-4.

Supplementary Material 1: Appendix 1. Calculation of Framingham Cardiovascular Risk Score.

Supplementary Material 2: Appendix 2. Standardized Toxicity Tables.

Acknowledgements
Not applicable.

Authors' contributions {31b}
MTH developed the original protocol and was the clinical lead investigator for stage 1; VMS wrote the manuscript and assumed the clinical lead after stage 1 completion; CB, CO, WS, ATC, and BT are trial physicians, and AS, IO, and KK are the study coordinators, all of whom reviewed and contributed to trial protocol revisions during the trial. MH and DLT provided clinical trial design advice; GJT provided protocol advice and coordination for sample collection, processing, and shipment; MY assessed and performed the statistical analyses for power and assessment.
MG and MP are the co-inventors of the vaccine product; MG is the principal investigator and scientific lead for the trial; MP provided vaccine manufacture oversight, quality control, and regulatory document preparation; MP, SR, AC, JD, VO, and AL developed and performed experimental protocols to assess clinical trial endpoints; CD and EK provided product development and commercial support.
All authors read and approved the final manuscript.

Funding {4}
This is an investigator-initiated trial, sponsored by the University of Alberta and the Li Ka Shing Institute of Virology (Canada). The trial and preliminary research are funded through the Canadian Institutes of Health Research (CIHR) Phase 1/2 Clinical Trials Grant program, the Heart Foundation (Australia); the Snow Foundation (Australia); the National Health and Medical Research Council of Australia; the National Foundation for Medical Research and Innovation (Australia); and the Lowitja Foundation (Australia).

Data availability {29}
The final trial data for this protocol can be supplied on request from the clinical lead (VMS) and scientific lead (MG) and approval from the Research Ethics Board.

Declarations

Ethics approval and consent to participate {24}
The University of Alberta Human Research Ethics Board (HREB) has assessed and approved this protocol as well as the consent form and all trial related documents—REB Pro00089919, ver 05Oct2023. Written, informed consent to participate will be obtained from all participants.

Consent for publication {32}
Not applicable. No identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial results. The participant information materials and informed consent form are available from the corresponding author upon request.

Competing interests {28}
MG and MP are co-inventors on the patents related to the vaccine candidates in trial.
The remaining authors declare that they have no competing interests.

Author details
[1]Department of Medicine/Division of Infectious Diseases, University of Alberta, Edmonton, AB, Canada. [2]Li Ka Shing Institute of Virology, University of Alberta, Edmonton, AB, Canada. [3]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada. [4]Department of Pediatrics, University of Alberta, Edmonton, AB, Canada. [5]Laboratory of Vaccines for the Developing World, Institute for Biomedicine and Glycomics, Griffith University, Nathan, QLD, Australia. [6]Clinical Trials Office, University of Alberta, Edmonton, AB, Canada. [7]Department Laboratory Medicine & Pathology, University of Alberta, Edmonton, AB, Canada. [8]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada. [9]Department of Medicine/Division of Cardiology, University of Alberta, Edmonton, Canada.

Received: 27 August 2024 Accepted: 12 November 2024
Published online: 19 November 2024

References

1. WHO. World Health Organization (15 December 2022). Disease outbreak news; increased incidence of scarlet fever and invasive group A Streptococcus infection - multi-country. 2022. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON429.
2. Ghamari SH, Abbasi-Kangevari M, Saeedi Moghaddam S, Aminorroaya A, Rezaei N, Shobeiri P, et al. Rheumatic heart disease is a neglected disease relative to its burden worldwide: findings from global burden of disease 2019. J Am Heart Assoc. 2022;11(13):e025284.
3. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis. 2009;9(10):611–6.
4. Excler JL, Kim JH. Accelerating the development of a group A Streptococcus vaccine: an urgent public health need. Clin Exp Vaccine Res. 2016;5(2):101–7.
5. Dale JB, Penfound T, Chiang EY, Long V, Shulman ST, Beall B. Multivalent group A streptococcal vaccine elicits bactericidal antibodies against variant M subtypes. Clin Diagn Lab Immunol. 2005;12(7):833–6.
6. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011;29(46):8175–8.
7. Pastural É, McNeil SA, MacKinnon-Cameron D, Ye L, Langley JM, Stewart R, et al. Safety and immunogenicity of a 30-valent M protein-based group a streptococcal vaccine in healthy adult volunteers: a randomized, controlled phase I study. Vaccine. 2020;38(6):1384–92.
8. Walkinshaw DR, Wright MEE, Mullin AE, Excler JL, Kim JH, Steer AC. The Streptococcus pyogenes vaccine landscape. NPJ Vaccines. 2023;8(1):16.
9. Pruksakorn S, Currie B, Brandt E, Martin D, Galbraith A, Phornphutkul C, et al. Towards a vaccine for rheumatic fever: identification of a conserved target epitope on M protein of group A streptococci. Lancet. 1994;344(8923):639–42.
10. Brandt ER, Hayman WA, Currie B, Carapetis J, Wood Y, Jackson DC, et al. Opsonic human antibodies from an endemic population specific for a

conserved epitope on the M protein of group A streptococci. Immunology. 1996;89(3):331–7.
11. Brandt ER, Hayman WA, Currie B, Pruksakorn S, Good MF. Human antibodies to the conserved region of the M protein: opsonization of heterologous strains of group A streptococci. Vaccine. 1997;15(16):1805–12.
12. Hayman WA, Brandt ER, Relf WA, Cooper J, Saul A, Good MF. Mapping the minimal murine T cell and B cell epitopes within a peptide vaccine candidate from the conserved region of the M protein of group A streptococcus. Int Immunol. 1997;9(11):1723–33.
13. Nordstrom T, Pandey M, Calcutt A, Powell J, Phillips ZN, Yeung G, et al. Enhancing vaccine efficacy by engineering a complex synthetic peptide to become a super immunogen. J Immunol. 2017;199(8):2794–802 (Baltimore, Md : 1950).
14. Turner CE, Kurupati P, Wiles S, Edwards RJ, Sriskandan S. Impact of immunization against SpyCEP during invasive disease with two streptococcal species: Streptococcus pyogenes and Streptococcus equi. Vaccine. 2009;27(36):4923–9.
15. Zinkernagel AS, Timmer AM, Pence MA, Locke JB, Buchanan JT, Turner CE, et al. The IL-8 protease SpyCEP/ScpC of group A Streptococcus promotes resistance to neutrophil killing. Cell Host Microbe. 2008;4(2):170–8.
16. Pandey M, Langshaw E, Hartas J, Lam A, Batzloff MR, Good MF. A synthetic M protein peptide synergizes with a CXC chemokine protease to induce vaccine-mediated protection against virulent streptococcal pyoderma and bacteremia. J Immunology. 2015;194(12):5915–25 (Baltimore, Md : 1950).
17. Pandey M, Mortensen R, Calcutt A, Powell J, Batzloff MR, Dietrich J, et al. Combinatorial synthetic peptide vaccine strategy protects against hypervirulent CovR/S mutant streptococci. J Immunol. 2016;196(8):3364–74 (Baltimore, Md : 1950).
18. NIH. Division of Microbiology and Infectious Diseases (DMID) adult toxicity table: National Institute for Allergy and Infectious Diseases. 2007. Available from: https://www.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/Documents/dmidadulttox.pdf.
19. Ozberk V, Reynolds S, Huo Y, Calcutt A, Eskandari S, Dooley J, et al. Prime-pull immunization with a bivalent M-protein and spy-CEP peptide vaccine adjuvanted with CAF(R)01 liposomes induces both mucosal and peripheral protection from covR/S mutant Streptococcus pyogenes. mBio. 2021;12(1):e03537–20. https://doi.org/10.1128/mBio.03537-20.
20. Sekuloski S, Batzloff MR, Griffin P, Parsonage W, Elliott S, Hartas J, et al. Evaluation of safety and immunogenicity of a group A streptococcus vaccine candidate (MJ8VAX) in a randomized clinical trial. PLoS ONE. 2018;13(7):e0198658.

Publisher's Note