SUPPLEMENTARY DATA

Safety and immunogenicity of novel group A streptococcal peptide vaccines targeting cryptic epitopes (J8-K4S2 and P*17-K4S2): a first-in-human, double-blinded, phase 1 randomised controlled trial

Vanessa Meier-Stephenson[1-3]*†, Manisha Pandey[6,7]*†, Simone Reynolds[6#], Victoria Ozberk[6#], Christie Short[6], Kelly Kim[7], Michael T. Hawkes[2,4,5], William Stokes[1,9], Catherine Burton[4,13], Alena Tse-Chang[4], Ainslie Calcutt[6], Zhuoqi Xiao[6], Chenglong Wang[6], Jessica Dooley[6], Scott Jamieson[8], Eloise Keeffe[6], Conar O'Neil[1], Ijeoma Ogbuehi[8], Avi Seth[8], Michael R. Batzloff[6], Joshua Osowicki[11], Andrew Steer[11], Ben Tyrrell[12], Chris Davis[6], Gregory J. Tyrrell[9], Maryna Yaskina[13], D. Lorne Tyrrell[2,3], Michael Houghton[2,3], Michael F. Good[2,3,6]†

*co-First authors; [#]co-Second authors; †corresponding authors.

Includes:

1. Additional detailing on the trial operations and AEs.
    a. Protocol deviations
    b. AE details
2. Supplementary data on immunogenicity
3. Trial Protocol

1. Additional detailing on the trial operations and non-significant AEs.

(a) Protocol deviations:

There was one protocol violation in the first stage with sentinel dosing, whereby a participant was enrolled with very mild mitral valve prolapse (MVP), deemed clinically insignificant and dosed with the p*17-K4S2, however, upon deeper scrutiny of exclusion criteria, any degree of MVP had been intended and so the individual was removed from further dosing. The individual experienced mild injection site erythema (Grade 1 AE), and completed all remaining trial follow-ups, including repeat echocardiograms without any clinical concern or further attributable AEs.

There were also several minor protocol deviations with regards to using laboratory-based creatinine instead of POC for non-dosing visits; as well as an initial protocol requirement for rotating between arms for injection (subsequently left to participant preference) – both of which led to protocol amendments. One participant's 2-week follow-up visit was 2 days out-of-window.

There was also a protocol deviation of note with the unblinding of the first participant of the stage 2 RCT portion of the trial, whereby pharmacy had provided the product label with delivery and the participant was dosed before the label was discovered by the coordinator. The study PI was unblinded by the coordinator while reporting the lapse. No further lapses in blinding occurred throughout the remainder of the trial.

(b) Additional AEs - non-attributable and/or non-significant:

Cardiac and renal AEs are reported separately for specific focus. A summary of the total number of AEs with grading; AE specific to reactogenicity and additional AEs by system are tabulated below (**Supplementary Tables 1-3**).

STAGE 1:

Cardiac AEs: Two participants had changes noted on echocardiograms – one (who received J8-K4S2) had trace tricuspid regurgitation (TR) on screening echo and after their second vaccine dose was graded as mild TR. This was discussed in detail with cardiology, and it was deemed within normal variation between echocardiograms with slightly differing volume status. The tricuspid valve was otherwise of normal structure and deemed to have no significant valvular dysfunction. The second participant (who received p*17-K4S2) had a suspicion of an echogenic mobile mass (7mm) attached to one of the chordae after the second dose of vaccine. Upon notification by the echocardiologist, the participant was informed and brought back in to ascertain any symptoms or clinical findings, of which there were none and physical exam and an extended blood panel were unremarkable. A repeat echocardiogram was also performed with extended evaluations and comparisons with review by two cardiologists, but the mobile mass was no longer visualized. Again, the participant reported no symptoms related to potential emboli and remained asymptomatic throughout. There was no further suspicion for emboli or endocarditis.

Renal AEs: Additional details and timeline of MCD case –

Additionally, there was one participant in the J8-K4S2 test arm that was enrolled with signs of protein detected on urinalysis (1.0g/L), initially deemed clinically insignificant, and with a normal point-of-care (POC) creatinine at time of dosing, the participant was cleared to receive the dose. The urinalysis collected on the day of dosing (and resulted afterwards) showed an increase in protein to 3.0g/L. Follow-up urinalysis on the participant 2 weeks later showed a return to 1.0g/L, and again on the day of dosing (1.0g/L) with normal POC creatinine. A 24-hr urine sample was collected and showed 1.18g/L protein. Two weeks later, urinalysis was negative for any protein. Urine was again collected on the day of dosing for the third dose, having a normal urinalysis within the week and normal POC creatinine. The urinalysis had resulted after dosing and was again 3.0g/L, prompting notification of the family physician and a nephrology consult. 24-hr urinalysis revealed nephrotic range proteinuria. A declining protein on repeat urines was reassuring, but nine weeks after the 3rd dose, the participant developed peripheral edema and presented to the ED. Nephrology arranged for a renal biopsy. The proteinuria was decreasing and became negative over the subsequent two weeks without intervention. The renal biopsy revealed minimal change disease (MCD). The case was reviewed in detail by the DSMB, particularly whether the MCD was deemed attributable or not given what is known of this condition's triggers with timing and ultimately deemed non-attributable. However, the situation prompted a protocol change, where urinalyses were required to be resulted and reported as normal and that no level of initial proteinuria would be deemed insignificant. The participant's symptoms and proteinuria resolved fully in the 3 months post-onset.

Other: See **Supplementary Tables 1-3**.

Supplementary Table 1. Stage 1 summation of AEs by Grading and attributability

Total AEs reported	**68**
Number of participants with AEs	**10**
Total SAEs reported1	**0**
Number of participants with SAEs1	**0**
Number of AEs by Toxicity Grading	
Grade 1	**15**
Grade 2	**8**
Grade 3	**1**
Grade 4	**0**
N/A	**44**
Participants with AEs by Toxicity Grading	
Grade 1	**7**
Grade 2	**4**
Grade 3	**1**
Grade 4	**0**
N/A	**10**
Number of AEs by Relationship to Drug[1]	
No	**33**
Yes	**5**
Possibly	**28**

[1]Relationship assessment is missing for 2 AEs

Supplementary Table 2. Stage 1 participants reactogenicity. Open-label collection of AEs.

	J8-K4S2	**p*17-K4S2**
Pain at injection site	1	3
Swelling	0	0
Redness	0	1
Itching	0	0
Fever	3	0
Chills/sweats	1	0
Headache	1	3
Dizziness	0	0
Fatigue	1	0
Malaise	0	0
Myalgia	2	0
Arthralgia	1	0
Totals	**11**	**7**

Supplementary Table 3. Stage 1 participant AEs by system.

		J8-K4S2	p*17-K4S2
Cardiac			
	Bradycardia	0	1
	Arrythmia	0	1
	TV regurgitation	1	0
	TV thickening	0	1
Respiratory			
	Cough	1	0
	COVID-19	0	1
	Bronchitis	1	0
Gastrointestinal			
	Dysgeusia	0	2
	Constipation	0	1
Urinary			
	Urinary infection	2	1
	Urinary leukocytosis	1	2
	Proteinuria	3	0
	Hematuria	5	2
	Elevated creatinine	0	0
	Calcium oxalate crystals	1	0
Laboratory abnormalities			
	Anti-DNase test pos	1	0
	Elevated glucose	0	1
	Elevated neutrophils	0	1
	Decreased neutrophils	0	2
	Elevated CK	0	1
	Anemia	2	0
	Hypoalbuminemia	1	0
	Hypokalemia	0	2
Other			
	Ankle sprain	1	0
	Peripheral edema	1	0
	“Brain fog”	1	0
TOTALS		**22**	**19**

STAGE 2:

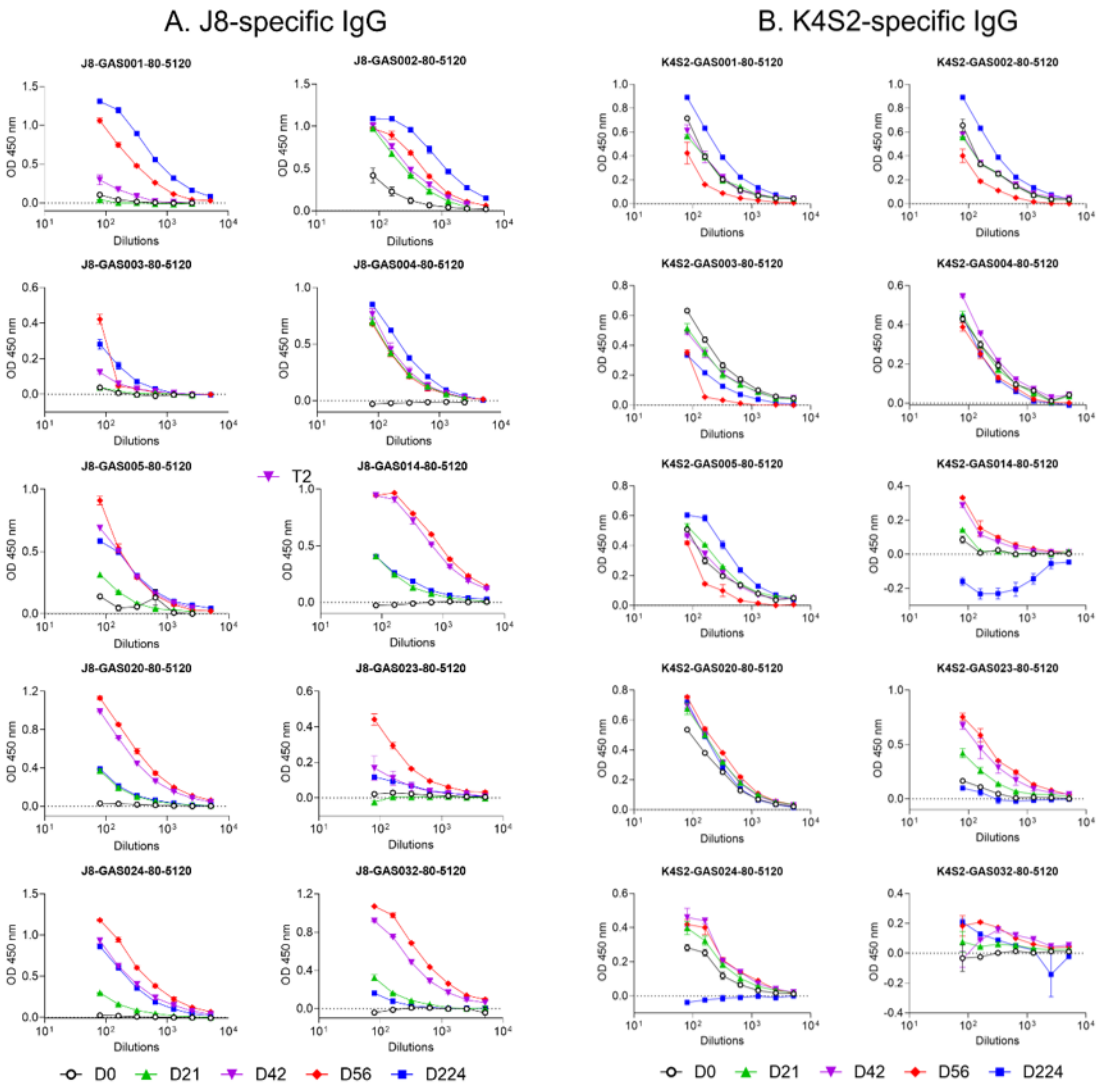
Cardiac AE: Detailed in the main text.

Renal AEs: Transient proteinuria was reported in two participants (control arm); and transient hematuria in eight participants (four in the control arm; three in J8-K4S2; and one in p*17-K4S2), seven of whom were menstruating; none triggered additional investigations.

Other: See **Figure 2**, main text.

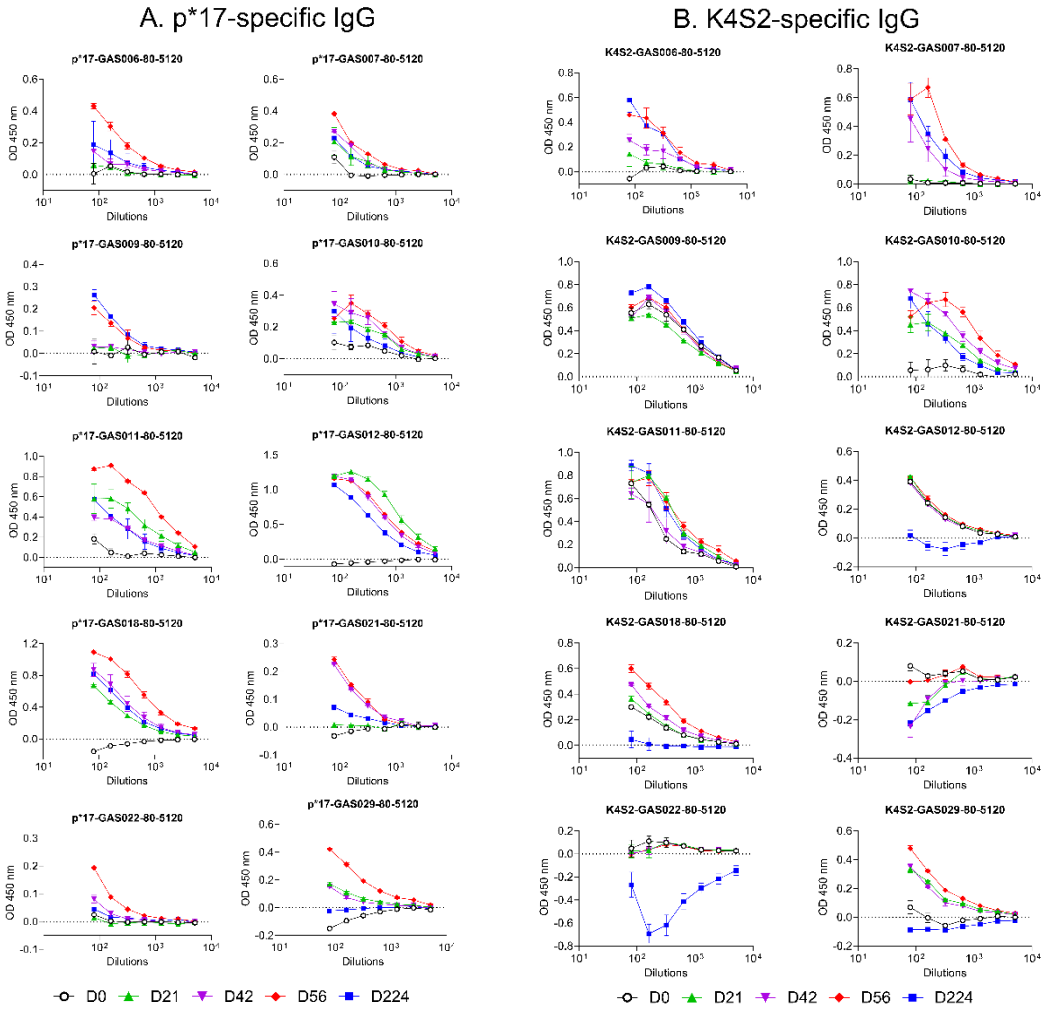
2. Supplementary Figures

Supplementary Figure 1.



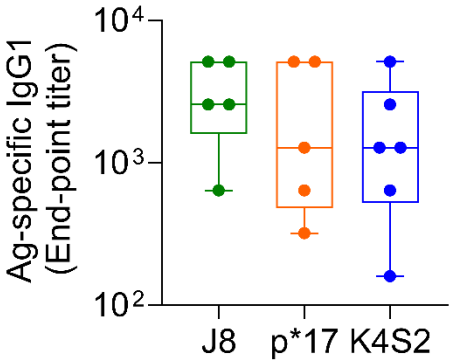
Supplementary Fig. 1. Assessment of vaccine antigen-specific IgG in sera samples from J8-K4S2 cohort participants. Ten participants (GAS001-GAS005, GAS014, GAS020, GAS023, GAS024 and GAS032) were vaccinated intramuscularly with J8-CRM+K4S2-CRM/Alum on days 0, 21 and 42. Serum samples were collected prior to vaccination (D0), three weeks (±7 days) after each vaccination (D21, D42 and D56), and six months after the final vaccination (D224). Antigen-specific IgG responses to (A) J8 and (B) K4S2 were measured by ELISA. OD450 readings for sera dilutions between 1:80 and 1:5120 are shown. Data were background-corrected by subtracting OD values obtained from plates without antigen and are presented as the mean of technical replicates. Negative values following background correction reflect very low antigen-specific IgG levels relative to background signal in the no-antigen control wells.

Supplementary Figure 2.



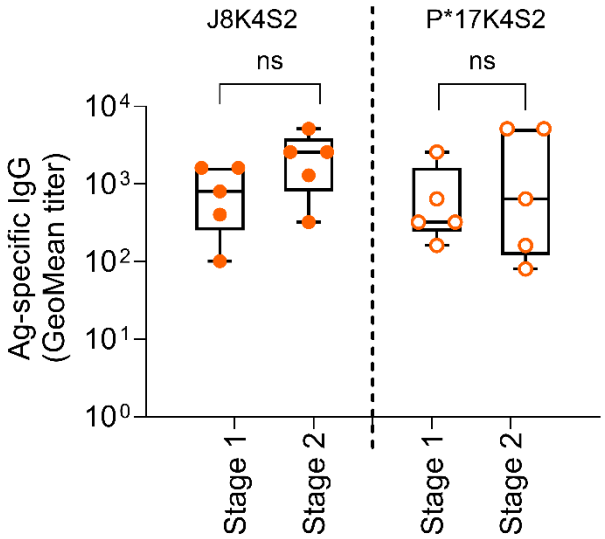
Supplementary Fig. 2. Assessment of vaccine antigen-specific IgG in sera samples from P*17-K4S2 cohort participants. Ten participants (GAS006, GAS007, GAS009, GAS010, GAS011, GAS012, GAS018, GAS021, GAS022 and GAS029) were vaccinated intramuscularly with p*17-CRM + K4S2-CRM/Alum on days 0, 21 and 42. Serum samples were collected prior to vaccination (D0), three weeks (±7 days) after each vaccination (D21, D42 and D56), and six months after the final vaccination (D224). Antigen-specific IgG responses to **(A)** p*17 and **(B)** K4S2 were measured by ELISA. OD450 readings for sera dilutions between 1:80 and 1:5120 are shown. Data were background-corrected by subtracting OD values obtained from plates without antigen and are presented as the mean of technical replicates. Negative values following background correction reflect very low antigen-specific IgG levels relative to background signal in the no-antigen control wells.

Supplementary Figure 3.



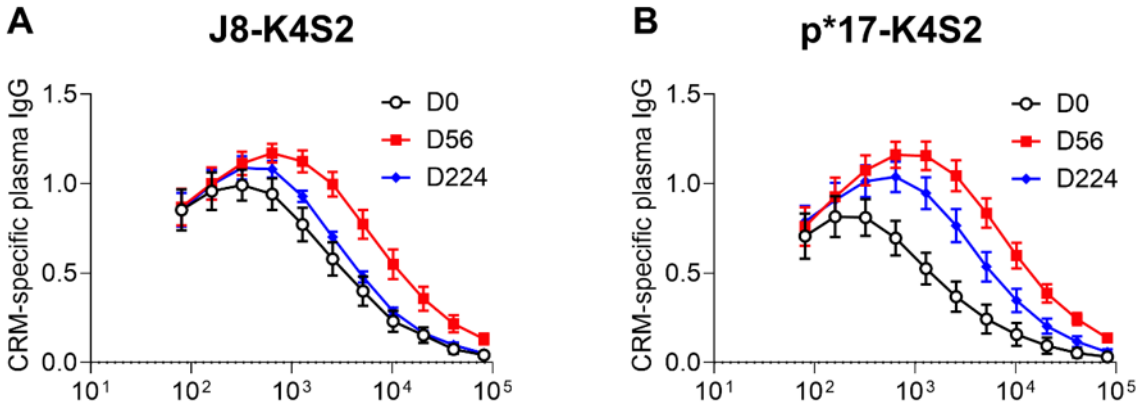
Supplementary Fig. 3. Antigen-specific IgG1 responses following vaccination with J8-CRM+K4S2-CRM/Alum or p*17-CRM+K4S2-CRM/Alum. Participants received intramuscular vaccinations on Days 0, 21, and 42 (n =10 per cohort). Antigen-specific IgG1 responses were measured by ELISA in a subset of Day 56 serum samples for which sufficient serum volume was available, and endpoint titres were calculated. J8- and p*17-specific titres are shown for their respective vaccine cohorts, while K4S2-specific titres are shown for a subset of participants from both cohorts (n = 3 per cohort).

Supplementary Figure 4.



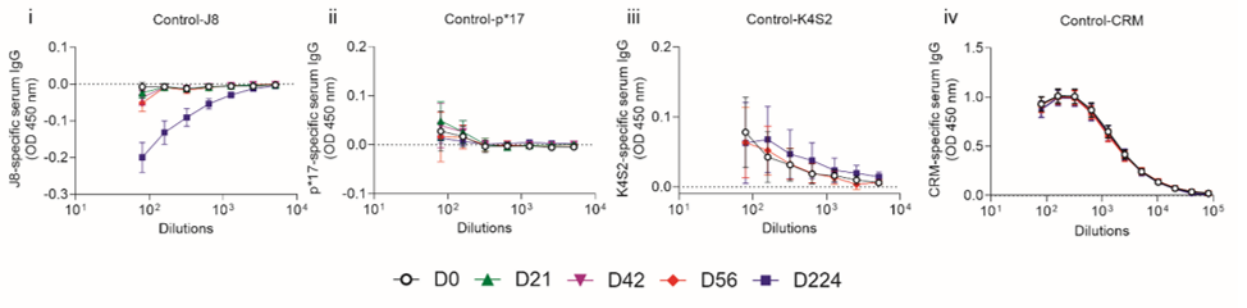
Supplementary Fig. 4. Immune response comparison between Stage 1 and Stage 2 trials. The trial was conducted in two stages. Stage 1 was an open-label sentinel safety test dosing phase with ten participants (n=5 per vaccine cohort). Stage 2 was a double-blind randomised controlled trial (RCT) involving twenty participants, evaluating two vaccine candidates against a comparator rabies vaccine (RabAvert) (n=5 per vaccine cohort and n=10 in the RabAvert cohort). Participants in both stages were vaccinated intramuscularly on days 0, 21, and 42. Sera were collected before vaccination (D0), at three weeks ± 7 days post each vaccination (D21, D42, and D56), and six months after the final vaccination (D224). Sera were tested via ELISA to assess vaccine antigen-specific IgG antibody responses. Optical density was measured at 450 nm, and mean titers for each participant were calculated from duplicate measurements using an OD cut-off of 0.112. Data comparing the geomean titers from Stage 1 and Stage 2 after final immunization (D56) are shown. Statistical analyses were performed using a two-tailed Mann–Whitney test, with significance indicated when $p > 0.05$; (ns) indicates non-significant differences.

Supplementary Figure 5.



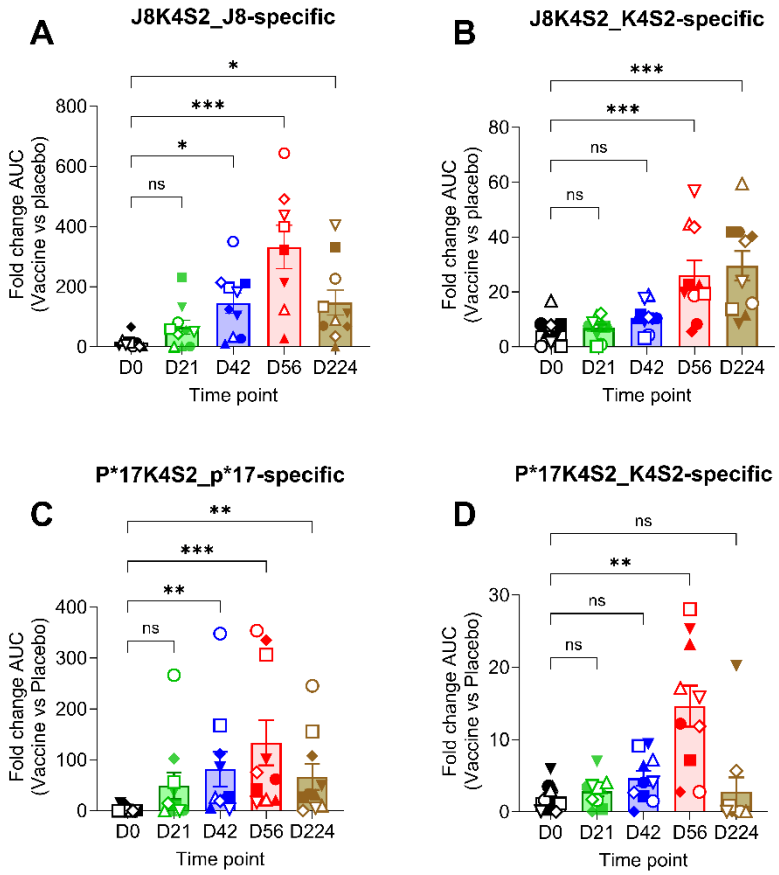
Supplementary Fig. 5. Assessment of CRM-specific IgG in sera samples from vaccinated participants. Participants were vaccinated intramuscularly with either J8-CRM+K4S2-CRM/Alum or p*17-CRM+K4S2-CRM/Alum on days 0, 21 and 42 (n = 10 per cohort). Serum samples were collected at designated time-points and CRM-specific IgG were determined by ELISA. CRM197-specific IgG responses (OD450) at before vaccination (D0), and after final vaccination (D56, and D224) for the (**A**) J8-K4S2, and (**B**) P*17-K4S2, cohorts are presented as mean ± SEM.

Supplementary Figure 6.



Supplementary Fig. 6. **Assessment of vaccine antigen-specific IgG in sera samples from RabAvert control participants.** Ten participants were vaccinated intramuscularly with RabAvert on D0, 21 and 42 in parallel with the StrepA vaccine recipients. Serum samples were collected before vaccination (D0), three weeks (±7 days) after each vaccination (D21, D42, and D56), and six months after the final vaccination (D224). The samples were tested for IgG against vaccine antigens by ELISA in a blinded manner. IgG response to **(i)** J8, **(ii)** p*17, and **(iii)** K4S2 at D0, D21, D42, D56, and D224 are presented as mean ± SEM. **(iv)** CRM197-specific IgG responses are presented for D0, D56, and D224. Data were background-corrected by subtracting OD values obtained from plates without antigen and are presented as the mean of technical replicates. Negative values following background correction reflect very low antigen-specific IgG levels relative to background signal in the no-antigen control wells.

Supplementary Figure 7.



Supplementary Fig. 7. Area under the curve (AUC) comparison for the vaccinated and control cohort. Participants were vaccinated intramuscularly with either J8-CRM+K4S2-CRM/Alum, p*17-CRM+K4S2-CRM/Alum in Stages 1 and 2 (n=5 per stage) or control vaccine RabAvert (Stage 2 only, n=10) on days 0, 21 and 42. Serum samples were collected before vaccination (D0), three weeks (± 7 days) post each vaccination (D21, D42 and D56), and 6 months after the final vaccination (D224). Sera were tested in ELISA to assess vaccine antigen-specific IgG antibody responses as described in Supplementary Figure 1. AUC values were calculated from titration curves (1:80-1:5120) following background correction using plates without antigen. For each vaccinated participant, fold-change was determined relative to the geometric mean AUC of the control cohort. Fold-change in AUC is shown for (**A**) J8-specific IgG responses in the J8-K4S2 cohort, (**B**) p*17-specific IgG responses in the p17-K4S2 cohort, (**C**) K4S2-specific IgG responses in the J8-K4S2 cohort, and (**D**) K4S2-specific IgG responses in the p17-K4S2 cohort. For descriptive immunogenicity analyses, participants from Stages 1 and 2 are presented together by vaccine candidate (n=8-10). Statistical analyses of repeated measurements were performed using the Kruskal-Wallis test with Dunn's multiple-comparisons test, with correction for multiple comparisons. Statistical significance is indicated as *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.005$, and ****$p \leq 0.001$; ns, not significant.

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

PROTOCOL IDENTFYING NUMBER PRO 00089919

PROTOCOL VERSION DATE: Oct 5, 2023

Registered Clinicaltrials.gov Identifier: NCT04882514

QUALIFIED INVESTIGATOR:

Dr. Vanessa Meier – Stephenson (MD., PhD., FRCPC.)

Address: Rm 1-124P Clinical Science Building, 11350 83 Ave

Edmonton, AB, T6G 2G3

Telephone: 780-492-6942

Signature of Investigator:

I agree to the terms of this protocol and all amendments. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and national, provincial, and local regulations.

_____

(printed name) **(signature)** **(date)**

SPONSOR:

The Governors of the University of Alberta

2-51 South Academic Building

Edmonton, AB. T6G 2G7

STUDY MONITORING:

Quality Management in Clinical Research (QMCR)

400 College Plaza

8215 112 St.

Edmonton, AB. T6G 2C8

TABLE OF CONTENTS

PROTOCOL SUMMARY

Title	**A Phase 1 Clinical Trial of a Peptide-Based Group A Streptococcal Vaccine**
Short Title	*GAS Vaccine*
Protocol Number	*PRO 00089919*
Phase	*1*
Methodology	*Stage 1: Test doses - ten (10) participants* *Stage 2: Controlled, double-blind RCT – twenty (20) participants*
Study Duration	*Estimated study duration from first participant to last participant:* *Approx. 18 months*
Study Center(s)	1. *University of Alberta Hospital Clinical Investigations Unit* 2. *NACTRC*
Objectives	1. *Monitor and document possible adverse events occurring in healthy volunteers receiving Strep A vaccine, including cardiac valve assessment (echocardiogram).* 2. *Measure serum antibody titres before and after 1, 2, and 3 doses of Strep A combination peptide vaccines, and compare titres 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.*
Number of Participants	*30 participants*
Diagnosis and Main Inclusion Criteria	*The study will enroll healthy adult participants between the ages of 18-45 (inclusive) with no evidence of underlying heart disease.*

Study Product, Dose, Route, Regimen	1. *p*17-CRM$_{197}$ (25µg) + K4S2-CRM$_{197}$ (6.25µg): TOTAL 31.25 µg* 2. *J8-CRM$_{197}$ (50µg) + K4S2-CRM$_{197}$ (6.25µg): TOTAL 56.25 µg* 3. *Comparator (rabies) vaccine (RABAVERT)* *Intramuscular injection administered at 0, 3, and 6 weeks*
Duration of administration	*Participants will remain in the study for a total of 10 months (including follow-up visits)*
Statistical Methodology	*The primary endpoint is the frequency of adverse events in each study arm (descriptive statistics).*

GENERAL INFORMATION

1.1 Name and title of person authorized to sign the protocol and the protocol amendments for sponsor:

1. *Name:* Vanessa Meier-Stephenson, MD, PhD, FRCPC
   Title: Assistant Professor, Department of Medicine
   Address: **Rm 1-124P** Clinical Sciences Building, 11350 83 Ave.T6G 2G3
   Telephone: 780-492-6942

1.2 Medical experts for the trial

1. *Name:* Vanessa Meier-Stephenson, MD, PhD, FRCPC
   Title: Assistant Professor, Department of Medicine
   Address: 1-131 Clinical Sciences Building, 11350 83 Ave.T6G 2G3
   Telephone: 780-492-6942
2. *Name:* Michael Hawkes, MD, PhD, FRCPC
   Title: Associate Professor, Department of Pediatrics
   Address: 3-593 ECHA, 11405 87 Ave, Edmonton, AB, T6G 1C9
   Telephone: 780-492-8038
3. *Name:* Alena Tse-Chang, MD, FRCPC
   Title: Assistant Professor, Department of Pediatrics
   Address: 3-557 ECHA, 11405 87 Ave, Edmonton, AB, T6G 1C9
   Telephone: 780-492-8038
4. *Name:* Catherine Burton, MD, MSc, FRCPC
   Title: Assistant Professor, Department of Pediatrics
   Address: 3-537 ECHA, 11405 87 Ave, Edmonton, AB, T6G 1C9
   Telephone: 780-492-8038
5. *Name*: William Stokes, MD, MSc, FRCPC
   Title: Assistant Professor, Department of Pathology and Laboratory Medicine
   Address: 2B4.58 WMC, 8440-112 St NW, Edmonton, AB, T6G 2J
   Telephone: 780-720-702
6. *Name*: Conar O'Neil, MD**,** MSc, FRCPC
   Title: Associate Professor, Department of Medicine, Division of Infectious Diseases
   Address: WMC 2B4.58, 8440-112 St, Edmonton, AB, T6G 2J2
   Telephone: 780-735-9192
7. *Name*: Benjamin D. Tyrrell MD, FRCPC

Title: Assistant Professor, Division of Cardiology

Address: Suite 350, East Tower Hys Centre, Edmonton, Alberta T5H 4B7

Telephone: (780) 428-3246

1.3 Principal Investigators:

1. Prof. Michael Good, MD, PhD, Adjunct Professor, Department of Medical Microbiology and Immunology, University of Alberta
2. Dr. Gregory Tyrrell, PhD, Professor, Division of Diagnostic and Applied Microbiology, Department of Laboratory Medicine & Pathology, University of Alberta and Provincial Laboratory for Public Health, Alberta Health Services
3. Dr. Michael Hawkes, MD, PhD, Departments of Pediatrics and Medical Microbiology and Immunology, University of Alberta
4. Dr. Vanessa Meier-Stephenson, MD, PhD, Department of Medicine, University of Alberta
5. Dr. David Lorne Tyrell, MD, PhD, Department of Medicine, University of Alberta
6. Dr. Michael Houghton, PhD, Department of Medicine, University of Alberta
7. Dr. Alena Tse-Chang, MD, Department of Pediatrics, University of Alberta
8. Dr. Catherine Burton, MD, MSc, Department of Pediatrics, University of Alberta
9. Dr. Benjamin Tyrrell, MD, Division of Cardiology, University of Alberta
10. Dr. William Stokes, MD, MSc, Department of Pathology and Laboratory Medicine, University of Alberta
11. Dr. Conar O'Neil, MD, MSc, Department of Medicine, University of Alberta

1.4 Co-Investigators:

1. Dr. Manisha Pandey, PhD, Institute for Glycomics, Griffith University
2. Dr. Simone Reynolds, PhD, Institute for Glycomics, Griffith University
3. Dr. Maryna Yaskina, PhD, Senior Biostatistician, Women and Children's Health Research Institute

1.5 Physician responsible for all trial-site related medical decisions

Name: Vanessa Meier-Stephenson, MD, PhD

Assistant Professor, Department of Medicine

Rm 1-124P Clinical Sciences Building, 11350 83 Ave.T6G 2G3

780-492-6942

Trial Sites:

1. Clinical Investigation Unit, University of Alberta Hospital, 2C3

   Tel: 780-407-6135

2. The Northern Alberta Clinical Trials and Research Centre (NACTRC)

400, 8214 112 St Edmonton, Alberta

T6G 2C8

1.6 Laboratories and other institutions involved in the trial

1. Alberta Precision Laboratories, University of Alberta
2. Dr. Gregory Tyrrell Research Laboratory, Division of Diagnostic and Applied Microbiology
3. Laboratory for Vaccines of the Developing World, Institute for Glycomics, Griffith University

2 BACKGROUND INFORMATION

Group A streptococci (Strep A) or *Streptococcus pyogenes*, is a major pathogen of humans causing an estimated 500,000 deaths worldwide each year. Worldwide, there are estimated to be over 600 million cases of streptococcal skin disease and 100 million cases of streptococcal pharyngitis each year. Untreated, Strep A infection can lead to the serious post streptococcal pathologies of rheumatic fever/rheumatic heart disease and post-streptococcal glomerulonephritis. People, primarily children, in all countries suffer from streptococcal pathology. Rheumatic heart disease is an important cause of acquired heart disease in developing countries.

Diseases caused by Strep A range from mild pharyngitis to skin infection with the most severe being necrotizing fasciitis, septicemia and in rare cases streptococcal toxic shock. Post infectious streptococcal sequelae can also occur with the two major manifestations being rheumatic fever and acute glomerulonephritis. These last two sequelae are thought to arise as the result of an autoimmune response against different Strep A proteins.

A major virulence factor for Strep A is the M protein, a coiled-coil alpha helical protein. The amino terminal specific segment of the M protein defines the serotype. The M protein is encoded by the *emm* gene and there are greater than 230 *emm* types. Serotype-specific immunity develops to Strep A but each strain of Strep A expresses only one type of M protein and there is limited cross-strain specific immunity. There are geographic variations in predominant circulating Strep A serotypes.

There are several approaches currently being explored by Strep A vaccine researchers [1]. Two strategies are briefly discussed. One approach involves combining epitopes representing the most common serotypes of Strep A circulating in the US [2, 3]. This approach, developed by Jim Dale and colleagues from the University of Tennessee, has reached Phase II clinical trials. This group's 30-valent vaccine represents over 80% of Strep A strains circulating in the US; however, coverage against strains outside the US is far less, and as low as 19.3% (for skin disease in the Pacific region) in many countries most affected by streptococcal disease [4, 5]. An alternative strategy being developed by Novartis is largely proprietary knowledge but is based on combination of three and possibly more recombinant virulence factors that have each been rendered non-

pathogenic [6]. This approach is at a much earlier stage of development with limited pre-clinical data reported to date.

We have developed two Strep A vaccines based on a conserved minimal epitope from the surface M protein combined with a non-M protein virulence protein. We initially identified a 20-amino acid peptide, referred to as p145, from the C3 repeat region of the M protein [7]. Antibodies, raised in mice, in the presence of human neutrophils and complement were able to opsonize multiple strains of Strep A [8]. Furthermore, p145 purified antibodies taken from First Nations volunteers living in a streptococcal-endemic region of Australia were also able to opsonize multiple strains [9]. In order to define the minimal peptide epitope, a series of overlapping 12-mers (monomeric units) was constructed. Unfortunately, these 12-mers were not able to induce antibodies to the parent p145 peptide. We determined that this was due to a loss of structure of the peptide, which is displayed as an alpha helix in the parent M protein. Technology was developed to fold the 12 mers within a larger peptide specifically designed to maintain folding [10]. Using this approach we were able to define the minimal peptide, which was recognized by anti-p145 antibodies and which could induce anti-p145 antibodies. The peptide of interest, J8, consists of 12 amino acids from p145 flanked by 16 amino acids from a non-streptococcal protein, GCN4 (from yeast) [10]. It is a ‘chimeric’ peptide. To improve immune responses to the peptide in an outbred population (such as humans), we chemically conjugated J8 to diphtheria toxoid (DT) and adsorbed J8-DT to aluminum hydroxide (Alum). Because the peptide is of minimal size with only 12 amino acids of streptococcal sequence the chances of developing an autoimmune response as a consequence of vaccination are minimal. The first vaccine contains the J8 peptide.

Further development of the p145 peptide involving iterative rounds of amino acid substitutions within the minimal B cell epitope led to the designing of a p145 derivative. The modified peptide referred to as ‘p*17’ contains the critical antigenic residues of p145 that also maintained the helical fold. Bioinformatics and *in vitro* analysis found no cross-reactivity to human heart proteins [11]. P*17 is similar to J8, but in mouse studies it has been shown to induce mucosal and skin immunity following intramuscular immunization, whereas J8 induces immunity to only skin disease. The second vaccine contains the p*17 peptide.

To further improve vaccine efficacy, we have developed the vaccines to contain the M protein peptides in combination with a second epitope, called K4S2 (vaccine 1: J8/K4S2 and vaccine 2: p*17/K4S2). Mutation within the Strep A cluster of virulence responder/ sensor (CovR/S) results in upregulation of numerous Strep A virulence factors, including the serine protease, *S. pyogenes* cell envelope proteinase (SpyCEP) [12]. SpyCEP cleaves CXC chemokines, IL-8 (human), and KC and MIP-2 (mouse) responsible for attracting neutrophils to the site of infection. J8-specific Abs require neutrophils for opsonic phagocytosis and J8-DT was ineffective against highly virulent Strep A strains that have upregulated SpyCEP as a result of mutations within CovR/S [13]. This lack of vaccine efficacy was reversible by combining an inactive 553-aa recombinant fragment of SpyCEP with J8-DT [13]. Furthermore, we identified a 20-aa SpyCEP epitope. The epitope does not have any IL8 protease activity, but has maintained the ability to induce neutralizing and

protective antibodies [14]. These antibodies completely protected IL-8 from SpyCEP mediated proteolysis [14]. We demonstrated that vaccination with the SpyCEP epitope, in combination with the minimal M protein epitope vaccine, J8-DT, induces potent protection against skin and deep tissue infection with different CovR/S mutant Strep A strains with different *emm* types [14]. This has also been demonstrated for p*17-DT (unpublished). The SpyCEP epitope is called K4S2.

The choice of carrier protein (CRM_{197}) for vaccines is based on extensive data from vaccine trials and clinical experience demonstrating its safety and efficacy. Currently licensed conjugate vaccines contain one of five carrier proteins: (1) diphtheria toxoid (DT); (2) a genetically modified form of diphtheria toxin, cross-reacting material (CRM_{197}); (3) tetanus toxoid (T); (4) outer membrane protein complex (OMPC) of *Neisseria meningitidis*, and protein D (HiD) *Haemophilus influenzae*. All 5 carrier proteins increase immunogenicity to covalently linked sugars, but differ in the magnitude, rate, and avidity of the antibody response. Of note, differences between CRM_{197} and DT (used as the carrier protein in our previous human volunteer clinical trial) are as follows: DT is purified wild-type diphtheria toxin that is subsequently detoxified with formaldehyde, called diphtheria toxoid (DT). CRM_{197} is a more standardized, nontoxic variant of diphtheria toxin isolated from *Corynebacterium diphtheriae* C7 (β197) cultures. CRM_{197} has no enzymatic activity and toxicity because of a point mutation at amino acid position 52 that substitutes glycine with glutamic acid [15]. CRM_{197} is antigenically similar to diphtheria toxin but is nontoxic and has more lysyl side-chains available for conjugation [16]. CRM_{197} is the carrier protein of several conjugate vaccines approved by Health Canada and widely used in public health infant vaccination programs across the country: meningococcal vaccines (Menjugate® and Menveo®) and pneumococcal vaccine (Prevnar® 13). Examples of clinical studies demonstrating the safety and efficacy of CRM_{197} containing vaccines include those for Hib [17, 18], meningococcus [19], and pneumococcus [20].

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.

The working hypothesis is that vaccination will be safe and will lead to antibodies that are able to kill multiple strains of Strep A *in vitro*.

2.1 Description of Investigational Product

We have developed two synthetic peptide conjugate vaccines. The first vaccine contains the peptides J8 and K4S2 and the second vaccine contains the peptides p*17 and K4S2. Each peptide is chemically conjugated to the carrier protein CRM_{197} to enhance its immunogenicity (J8-CRM_{197}, p*17-CRM_{197}, and K4S2-CRM_{197}). In each of the vaccines, the two peptide conjugates will be mixed together (J8-CRM+K4S2-CRM and p*17-CRM+K4S2-CRM) in equal concentration and then adjuvanted with Alhydrogel (‘Alum’) (J8-CRM+K4S2-CRM/Alum and p*17-CRM+K4S2-CRM/Alum), henceforth referred to as J8-K4S2 and p*17-K4S2.

Preclinical data

(i) Mouse model of Strep A pyoderma. To test the efficacy of J8 and p*17 in preventing impetigo/pyoderma, we developed a skin challenge model in mice that closely reflects human inoculation. Mouse hair is shaved from the nape of the neck and a small abrasion is made. Subsequently, $1x10^6$ Strep A organisms (streptomycin-resistant) are applied to the abrasion and mice are caged individually to prevent grooming. The lesions that develop closely resemble human impetigo. Histological examination reveals an influx of neutrophils (Appendix 1, Figure 1). The K4S2-related increase in IL-8 associated with the J8-K4S2 or p*17-K4S2 combination vaccines will result in more chemotaxis and immune cell infiltration, a major advantage of the combination vaccines.

(ii) Vaccine-mediated protection. J8 and p*17 have been tested for efficacy in different mouse models of disease. Immunization of mice subcutaneously or intramuscularly with 30µg J8 or p*17 conjugated to diphtheria toxoid or to CRM_{197} results in the production of peptide-specific antibodies that can opsonize multiple strains of Strep A *in vitro* with human blood as a source of neutrophils and complement.

Vaccinated mice that are challenged with Strep A either via the intraperitoneal route or via the skin (see above) are protected from death due to sepsis. Our published data show similar degrees of protection following skin challenge (Appendix 1, Figures 2 and 3 [11, 13]). In these experiments, vaccinated and control mice (vaccinated with PBS/Alum) were challenged with $1x10^6$ organisms of different strains of Strep A isolated from patients. These organisms were of different M serotypes. Four weeks after a primary and two booster injections, there was a dramatic reduction in the colony counts in both blood and skin in the J8 or p*17 vaccinated mice compared to the PBS/Alum immunised controls.

Pilot data for a Phase I trial of J8-DT vaccine

Ten volunteers were selected for a Phase I double-blinded trial conducted at Q-Pharm (Australia). Eight volunteers received a single dose of 50µg of J8-DT/Alum intramuscularly into the deltoid muscle. Two volunteers received an injection of PBS/Alum. The study was double blinded. Prior to the trial, volunteers were selected based on a number of criteria, including age (18-45yrs); absence of any history of heart disease or rheumatic fever; absence of pre-existing serum antibodies to J8; absence of any echocardiogram-demonstrated heart valve abnormalities as determined by a cardiologist; absence of any history or biochemical determination of substance abuse. Following vaccination, the volunteers were monitored closely for any adverse events, but none were recorded. They were examined monthly by a cardiologist for the presence of any cardiac valve abnormalities. Sera and lymphocytes were collected for immunological assessment. The serum antibody responses to the J8 peptide were measured by ELISA. The antibody response data (Appendix 1, Figure 4 and 5) show all of the 8 individuals who received the J8-DT/Alum vaccine responded by the production of J8-specific antibodies at 28 days post vaccination [21]. The two control individuals who received the PBS/Alum did not generate J8-specific antibodies. By 6 months post-vaccination the J8-specific antibody levels had declined to near baseline in most individuals.

2.2 Potential risks and benefits

Risks

This is a Phase I trial and safety is the paramount issue. In preparation for this trial, an extensive toxicological assessment, in rats, was performed on both vaccines by Citoxlab, USA. Acute toxicology and tissue-specific (skin, heart, brain, and kidney) toxicology assessments determined that there were no adverse events in rats following vaccination with either vaccine.

Prior to the initial pilot Phase I trial of the J8-DT vaccine, we were particularly concerned about the risk of inducing rheumatic fever. Our pre-clinical development strategy for the vaccine was focused on eliminating this risk, which was achieved by designing an absolutely minimal vaccine (only 12 amino acids in the vaccine represent a Strep A sequence). The pilot Phase 1 trial was funded by the US NIH who also provided oversight for the study. In preparation for the trial, general toxicological assessment was undertaken in rabbits by the IIT Research Institute (IITRI), USA. The toxicology report was reviewed by the Human Research Ethics Committee of the Queensland Institute of Medical Research. In parallel, stability of the formulation was undertaken by several organizations including TetraQ (University of Queensland), Auspep (Melbourne) and Griffith University. In addition, safety testing was undertaken by Prof. N. Ketheesen (James Cook University, Australia) who is expert in a rheumatic heart disease model of rats (Lewis rat model). In brief, rats susceptible to streptococcal-mediated rheumatic heart disease, were vaccinated with J8-DTand a Veterinary Pathologist, blinded to the groups, reported on the histology for the study. The conclusion from the study was there was no evidence or observation of valvulitis or myocarditis following vaccination of rats (Figure 6, [22]). These pre-clinical data demonstrate the safety of J8 peptide. The results for these studies are published [23].

The J8-DT vaccine was administered as a single dose to 8 adult human volunteers in February 2013 (described above). Two other individuals received a placebo vaccine (PBS). There have been no adverse events and J8-specific antibodies were induced in all eight who received J8-DT (Appendix 1, Figure 4) [21]. These are the same 8 volunteers described under section 2.1 above.

In the designing of the p*17 peptide considered effort was made to ensure that the peptide was not cross reactive with human tissues. A bioinformatic analysis of the homology of p*17 to human proteins and *in vitro* investigations of binding of vaccine induced antibodies to human heart proteins was performed. Both investigations determined p*17 had no cross-reactivity with or significant homology to human proteins (Appendix 1, Figure 5, [11]).

This work with J8-DT demonstrates the pre-clinical safety in a rat valvulitis model, and an initial Phase 1 safety study in healthy human volunteers. The current protocol examines the J8-K4S2 and p*17-K4S2 combination vaccines, in which both peptides are covalently linked to a different carrier protein, CRM_{197}. We have established the pre-clinical efficacy of these combination vaccines [11, 13, 14], and we expect it to be equally safe, since the

K4S2 epitope and SpyCEP do not play a role in the pathogenesis of rheumatic fever, and CRM_{197} is a standard carrier protein widely used in public vaccination programs (e.g., meningococcal and pneumococcal vaccines in Canada).

We do not anticipate any serious adverse events (requiring hospitalisation) in this trial. At most there may be a low incidence (up to 20%) of mild pain or redness at the injection site, or low-grade fever and flu-like symptoms, such as asthenia, fatigue, headache, myalgia, and malaise; arthralgia, dizziness, lymphadenopathy, nausea, and rash requiring nothing more than acetaminophen. We do not expect any Arthus-type reaction given the low dose of CRM_{197} present in the vaccine.

Benefits

The benefit to trial participants is potentially protection from a common and sometimes severe infection, Strep A, if the vaccine proves to be effective. There is also the possibility that the trial participants will derive no individual benefits if the vaccine is ineffective.

Alternatively, there is great potential benefit to the global community of developing an effective vaccine against Strep A. The burden of illness in Alberta is substantial and is greater in First Nations (FN) compared to non-First Nations (NFN) people. In 2011, FN individuals comprised 4% of the population of Alberta (148,252 vs 3,665,555 NFN) but were disproportionately represented among persons dying of Strep A: 17% of all streptococcal related deaths in Alberta occurred in First Nations peoples, with septicaemia being the commonest cause (AHS Report).

The results of this trial will be used to plan a larger Phase II trial in at risk communities both in Canada and internationally. A study in Melbourne will involve a human challenge component to assess efficacy in a small population before large Phase II studies. During this Phase I study, we will engage Indigenous leaders as key stakeholders for possible future studies in First Nations communities. Internationally, we intend to take the results of this study to make the case for trials in paediatric populations. The greatest burden of Strep A pathology is in developing countries. We are contemplating a Phase II trial in Uganda, where our team (Dr. Hawkes) has deep-rooted community collaborations and extensive experience in conducting clinical trials. We have excellent contacts in Australia, New Zealand and in Fiji (e.g. Prof. J. Carapetis, Director of the Telethon Institute for Child Health Research, Perth; and Dr. Andrew Steer, Murdoch Children's Medical Research Institute, Melbourne), countries where streptococcal disease is highly endemic and rheumatic heart disease is a major public health concern, particularly amongst Indigenous peoples.

2.3 Justification of route, dosage, dosage regimen, and treatment period

The standard route of administration of conjugate vaccines (e.g., pneumococcal, meningococcal, Hib) is intra-muscular.

A phase 1 study of a comparable J8 peptide vaccine (J8-DT) used a dose of 50µg IM and demonstrated a robust antibody response [21]. We will use this same dose of J8 in the

combination peptide vaccine, J8-K4S2 (50µg J8-CRM_{197} + 6.25µg K4S2-CRM_{197}). We will also use the more potent p*17 peptide at half the dose (25µg J8-CRM_{197} + 6.25µg K4S2-CRM_{197}), to test if lower doses of 25µg may be immunogenic.

Of note, the previous J8-DT pilot study used a single dose of 50 µg of peptide conjugate [21]. This vaccine induced elevated levels of antibody that lasted 1 month. We now propose a three-dose regimen, consistent with other childhood conjugate vaccines, to increase the durability of the antibody response. We have demonstrated that three doses are protective against skin infection in a murine model (Appendix 1, Figure 7).

2.4 Statement of compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- International Conference on Harmonization (ICH) guideline E6: Good Clinical Practice: Consolidated Guideline
- The study will also be conducted according to site specific standard operating procedures (SOPs), the protocol, and regulatory guidelines.

2.5 Description of the population to be studied

The study will enroll healthy adult participants with no evidence of underlying heart disease.

3 TRIAL OBJECTIVES

To test the safety and immunogenicity of two synthetic peptide combination Strep A vaccines.

Our specific aims are to:

1. Monitor and document possible adverse events occurring in healthy volunteers receiving Strep A vaccine, including cardiac valve assessment (echocardiogram).
2. Measure serum and salivary antibody titres before and after 1, 2, and 3 doses of Strep A combination peptide vaccines, and compare titres 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.

The working hypothesis is that vaccination will be safe and will lead to antibodies that are able to kill multiple strains of Strep A *in vitro*.

4 TRIAL DESIGN

4.1 Endpoints

Primary endpoint: Safety

1. Clinical symptoms and signs

2. Standard laboratory parameters (hematological and biochemical)
3. Echocardiogram (mitral regurgitation)

Secondary endpoints: Immunogenicity

1. Antibody titres
2. Antibody recognition of bacteria proteins in whole cell preparations (Direct binding to bacteria) *in vitro*
3. IL-8 chemokine protection assay

Primary outcome

We will document adverse events using standard toxicity tables, adapted from the National Institute for Allergy and Infectious Diseases (NIAID) Division of Microbiology and Infectious Diseases (DMID) toxicity tables [24]. These are reproduced in Appendix 4. Clinical, laboratory, and ancillary data which will be collected for safety monitoring are shown in Appendix 2, Table 1.

In order to maximize safety, we will conduct the study in two phases, with test doses administered to five 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 Appendix 3, Figure 8.

Stage 1: Test doses

We will verify the immediate safety of the J8-K4S2 and p*17-K4S2 vaccines using a test dose in 10 volunteers. With respect to J8-K4S2 vaccine, safety of one of the peptide components (J8) has previously been established [21]. Therefore, we will begin with a test dose of J8-K4S2. The first IM injection of J8-K4S2 will be administered to the first participant. We will wait two days, assess safety, then administer J8-K4S2 to a second participant after this time, if safety has been established. We will wait two days, assess safety, then administer J8-K4S2 to a third participant after this time, if safety has been established. This process will continue until 5 participants have received a test dose of J8-K4S2. If there are no safety concerns with the first 5 participants, we will have shown the preliminary safety of the K4S2 component. If the J8-K4S2 vaccine is well-tolerated, second and third doses of J8-K4S2 will be administered at 3 and 6 weeks, testing the safety of repeated dosing.

We will next administer p*17-K4S2 to a sixth participant, wait two days, assess safety, and administer p*17-K4S2 to a seventh participant after this time, if safety has been established. We will wait two days, assess safety, then administer p*17-K4S2 to an eighth participant after this time, if safety has been established. This process will continue until 5 participants have received a test dose of p*17-K4S2. If there are no safety concerns, we will have shown the preliminary safety of the p*17 component. If the p*17-K4S2 peptide vaccine is well-tolerated, second and third doses of p*17-K4S2 will be administered at 3 and 6 weeks, testing the safety of repeated dosing.

Safety checkpoints for Stage 1 are shown in Appendix 3, Table 2. 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 4). 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.

This may result in fewer than 3 experimental vaccine arms. In this case, randomization will occur to fewer groups. If sufficient doses of vaccine product are available, we will increase the number of participants in each remaining group, maintaining the same total number of participants (n=30).

Stage 2: Random allocation to experimental and comparator vaccines

This phase represents a prospective, parallel arm (3 arms), randomized, placebo-controlled, double-blind clinical trial. Twenty participants are expected to be randomized in stage 2.

Secondary outcomes

1. ***Antibody titres***

Samples of sera and saliva will be collected from the participants prior to each injection and at the scheduled clinic visits as indicated in Appendix 2. Sera will be stored, and assays will be conducted on batched samples after all sera have been collected.

The titre of vaccine specific antibodies will be determined using ELISA. The presence of vaccine-specific antibodies in all participants in the study will be monitored as well as antibodies to CRM_{197}. We will investigate the relationship between the titer of vaccine-

specific antibodies and the number of doses administered. In addition, titres will be compared to comparator (rabies) vaccine recipients.

Standard ELISA will be performed to determine the level of J8, p*17, and K4S2 peptide-specific IgG concentrations in sera and antibody levels in saliva of all participants at the described time intervals. The assays produce objective, quantitative data, and will be performed in a manner blinded to clinical trial arm.

2. Direct binding activity of antibodies in vitro

We will determine the recognition of Strep A proteins by the vaccine-induced antibodies, against different standard Strep A strains. The direct binding activity of the 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.

3. Chemokine protection assay

We will measure the ability of vaccine-induced antibodies to block cytokine proteolysis. 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 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).

4.2 Description of measures taken to minimize bias

(a) **Randomization**

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. The randomization code will remain blinded to the study team and coordinators. The randomization code will be included in the enrolment module for the trial. The randomization code will link the vial allocation number to the treatment assignment. Each participant enrolled in the trial is assigned to a vial allocation number after demographic and eligibility data have been entered into the system. The code list for emergency unblinding purposes will be kept in a secure place at the clinical site.

(b) **Blinding**

Stage 1 of the study will not be blinded to the study team but the participants will be blinded to the dose that they receive. For Stage 2 of the study, all personnel from the University of Alberta Hospital, and NACTRC will be blinded to the randomization scheme, except for the member(s) of the study team at the Hospital who is/are responsible for preparing the vaccine (and comparator vaccine) and the trial statistician. The vaccine will be shipped in labeled vials and stored at the study pharmacy. In a location in the clinical

trials unit or hospital where blinded study personnel are not present, the unblinded study pharmacist will prepare that study product for injection in pre-filled syringes. The syringes will not indicate the study product, to preserve blinding. The content of the syringes will, however, be visible through the transparent sheath. This way, the person administering the vaccine can see the graduations on the syringe, know the volume they are administering, see the rate they are administering the vaccine, and stay within their clinical practice guidelines. Documentation will be made in the study record indicating the date and time of study product administration. The participant, the study personnel who perform study assessments after product administration, investigators, data entry personnel at the sites, and laboratory personnel performing immunologic assays will also be blinded to treatment assignment. The unblinded member of the team will be instructed not to share treatment code information with the participant, University staff, or the NACTRC study team staff at the clinical site. In addition, the unblinded staff will not perform any other study related duties apart from the preparation of the study product.

4.3 Investigational treatment

Product: The final vaccine formulations are a sterile liquid suspension intended for intramuscular injection supplied in a single dose vial. Each 1.0 mL dose of the vaccine candidate contains p*17-K4S2 or J8-K4S2 and approximately 25 µl of aluminum hydroxide (alum). This amount of alum contains approximately 500 µg/mL elemental aluminum, as determined using USP method for determination of aluminum in adsorbed vaccines. The amount of elemental aluminum is below the upper limits recommended by the FDA (0.85 milligrams per dose if determined by assay or 1.14 milligrams if determined by calculation on the basis of the amount of aluminum compound added). The vaccine formulation also contains phosphate buffer, pH 6.8-7.5. The recommended storage condition for vaccine formulation is 5°C ± 3°C. The product will be stored in a calibrated, temperature monitored refrigerator. The vaccine candidate will be aliquoted in clear Type I glass vials in a total volume of 1.15mL ± 5%. This amount will be sufficient to aliquot a single dose injection of 1.0 mL. The content in the vaccine candidate vials must not be frozen. Any vials containing frozen vaccine contents must be discarded.

4.4 Expected duration of subject participation

Screening, enrollment, and administration of 3 doses of vaccine will take 10 weeks. An additional 6 months for follow-up (repeated testing for antibody durability) will also be required. The total duration is 10 months.

4.5 Biobanking of Samples (Optional)

The study will include an optional biobanking component which requires a separate consent signature if the participant agrees to participate.

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

Samples will be collected at every study visit from enrollment onwards.

4.6 Description of stopping rules/discontinuation criteria

Individual patients

Stopping rules for an individual participant will be as follows:

1. One or more SAEs or grade 4 AEs, based on standardized toxicity tables (Appendix 4), related to the study vaccine.
2. New, clinically significant mitral valve regurgitation defined at the discretion of the cardiologist and/or effective regurgitant orifice area of >10mm^2.
3. New onset definite or probable acute rheumatic fever (ARF).

In case of safety concern, we will hold further vaccine doses from an individual patient until a full assessment has been made. The DSMB chairperson will be notified and may recommend unblinding of the study vaccine, a meeting of the DSMB, and/or permanent discontinuation of the experimental vaccine series.

In addition, further doses of vaccine may be held at the discretion of the trial physician, or at the participant's own discretion. Participants are free at any time to remove their consent and withdraw from the study. In case the vaccine series is interrupted or discontinued, attempts will be made to retain the participant in the study, collect data and blood tests as per protocol, in order to minimize incomplete data for an intention-to-treat analysis.

Entire trial

Halting rules temporarily suspend study enrolment until a safety review is convened. The objective of this review will be to decide whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed.

Halting rules for this study will be:

1. One or more SAEs or grade 4 AEs, based on standardized toxicity tables (Appendix 4), occurring in any trial participant, related to the study vaccine.
2. New, clinically significant mitral valve regurgitation defined at the discretion of the cardiologist and/or effective regurgitant orifice area of >10mm^2 in any trial participant.
3. New onset definite or probable acute rheumatic fever (ARF) in one or more trial participant.

Safety review, interpretation of results and decisions about discontinuation of the trial will be made by the DSMB, using the suggested guidelines given here. The DSMB composition will be as follows: (1) content expert; (2) clinical trialist; and (3) statistician. 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 costs, 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.

4.7 Accountability procedures, including comparator vaccine

A comparator vaccine will be used (10 participants). The comparator vaccine used will be the commercially-available, Health Canada licensed rabies vaccine, RabAvert® (GlaxoSmithKline). The purpose of including a comparator vaccine arm is for comparison of safety and immunogenicity parameters to a control group. A previous Phase I trial of an experimental Strep A vaccine conducted in Canada (Canadian Center for Vaccinology in Halifax, Nova Scotia, Canada) included a comparator arm consisting of three licensed vaccines with aluminum as adjuvant: hepatitis A, hepatitis B and human papillomavirus vaccines [5]. We will use a similar strategy, using the licensed vaccine RABAVERT. Licensed vaccine products provide a good benchmark “standard” of safety and reactogenicity against which a novel vaccine product can be compared. Participants who are randomized to receive the control also stand to benefit from immunization against rabies with this strategy. Participants will be informed after conclusion of the study as to whether or not they received the comparator vaccine so that they will know whether or not they were vaccinated against rabies.

The rabies vaccine is administered in a three-dose schedule, similar to the experimental vaccine, allowing it to be administered in a blinded fashion. The recommended schedule for RABAVERT is three doses administered on days 0, 7, and 21. We will immunize trial participants randomized to the comparator vaccine arm (n=10) on days 0, 21, and 42. Because this alternative three-dose schedule respects the minimum interval between doses, the efficacy is expected to be equivalent to the standard three-dose schedule.

4.8 Maintenance of the trial treatment randomization codes and procedures for breaking codes

We will use a secure online randomization system in the REDcap system. Only persons authorized to enrol patients will be given password protected access to the system. A statistician will oversee the randomization code, keeping the information secure until the trial is closed, and data entry finalized. The code list for emergency unblinding purposes will be kept in a secure place at the clinical site.

4.9 Data to be included on the case report forms (CRF)

Screening form

Participants recruited to the trial will have a screening form completed, according to inclusion and exclusion criteria, listed below (Sections 5.1 and 5.2).

Enrolment 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 will include the following:

- Baseline antibodies (serum and saliva) to p145, p*17, J8, K4S2 and DT or CRM_{197}
- Complete blood count and differential
- Electrolytes and random glucose
- Creatinine
- Troponin
- Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)
- Liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT])
- Urinalysis
- Pregnancy Test (serum pregnancy test at screening, all other pregnancy tests will be urine dipstick)
- Throat Swab for culture and rapid antigen testing
- Echocardiogram
- Electrocardiogram (ECG)

Clinical visits

At each visit, participants will be assessed for any change in their medical status, including intercurrent illness, new medications or vaccinations, travel. A focused physical assessment will be performed. Bloodwork for safety monitoring will be obtained at selected visits (Appendix 2). 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.

Primary endpoint: Safety

At each visit, systematic and standardized toxicity tables (NIAID) will be used to grade adverse reactions at each participant visit. These tables provide a classification for severity of clinical (symptoms and signs) as well as laboratory parameters. These are provided in Appendix 4.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 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.

5.2 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 5). Risk factors include sex, age, systolic blood pressure (mm Hg), smoking status, body mass index (BMI, kg/m2), 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 five 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 leukaemia, lymphoma, or neoplasm;
14. Presence or suspicion of impaired immune system function. Currently receiving or having within the past three years received immunosuppressive therapy, including systemic steroids, ACTH or inhaled steroids in dosages that are associated with hypothalamic-pituitary-adrenal axis suppression, such as 1mg/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 > 29mm/m2 to BSA); left ventricular dysfunction (Ejection Fraction < 50%; left ventricular hypertrophy (LV wall thickness > 11mm); Right ventricular dysfunction or dilatation (Subjective assessment);
22. Cardiac Valves/Haemodynamic Findings: Clinically significant mitral regurgitation defined: at the discretion of the cardiologist and/or effective regurgitant orifice area of >10mm^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 < 5mm and not circumferential);

25. Pre-existing significant structural valve disease (for example, but not limited to bicuspid aortic valve regardless of haemodynamic 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+ (1.0g/L) or 3+ (3.0g/L) if not decreasing on subsequent U/A or explained by other features. Trace proteinuria or 1+ (0.3g/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 is 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, trifluopromazine, 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.

5.3 Subject withdrawal criteria (terminating trial treatment)

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

If a participant experiences any SAE or grade 4 AE thought to be causally related to the vaccine, further doses of the vaccine will be held, and the DSMB chairperson will be notified. 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 participants:**

If for any reason a participant does not complete the study, the reason will be entered on the 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.

(d) 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 (Appendix 3, Table 2). 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.

6 TREATMENT OF PARTICIPANTS

6.1 Description of intervention

Product: Participants will receive the investigational product as outlined in section 4.3 for stage 1 test dose part of the study. For the stage 2 random allocation portion of the study, participants will be randomized to receive the investigation product outlined in section 4.3 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} (50µg) + K4S2-CRM_{197} (6.25µg): TOTAL 56.25 µg
2. p*17-CRM_{197} (25µg) + K4S2-CRM_{197} (6.25µg): TOTAL 31.25 µg
3. Comparator (rabies) vaccine (RABAVERT)

Dosing schedule: 0, 3 and 6 weeks (3 doses)

Route: Intramuscular. The injections should alternate between the left and right deltoid for each injection.

Treatment period: 9 weeks

6.2 Co-administered treatments during the trial

The trial will enrol healthy participants, who will not require co-treatments. We will record any medications the patient is taking during the trial for unrelated conditions.

Other vaccines during the trial period. Given the ongoing COVID-19 pandemic and annual influenza activity during winter months in Edmonton, 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.

6.3 Procedures for monitoring subject compliance

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 minutes following immunizations to monitor for any immediate adverse reactions.

6.4 Specification of efficacy parameters

Immunogenicity of the Strep A vaccine candidate is the trial efficacy parameter (secondary endpoint - safety is the primary endpoint).

1. Antibody titres
2. Direct binding activity of antibodies *in vitro*
3. Protection of IL-8 from proteolysis

6.5 Methods and timing for assessing, recording, and analyzing efficacy parameters

The description of assays for the determination of immunogenicity is detailed above in section 4.1 (Secondary Endpoints). These assays will be performed in batch on cryopreserved serum samples obtained at the time of each clinic visit. The timing of the bloodwork is given in Appendix 2. Analysis will use standard statistical methods for longitudinal data (e.g., paired t-tests).

7 ASSESSMENT OF SAFETY

7.1 Specification of safety parameters

The following standard definitions are based on the International Committee on Harmonization Guideline on Clinical Safety Data Management.

Adverse Event (or Adverse Experience)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. The grading of AEs will follow standardized toxicity tables (Appendix 4).

Serious Adverse Event

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

- *results in death,*

- *is life-threatening,*
- *requires hospitalization or prolongs existing hospitalization, or*
- *results in persistent or significant disability/incapacity*

7.2 The methods and timing for assessing, recording, and analyzing safety parameters

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 4) [24]. The data, initially recorded on paper for the study file, will be entered into an electronic database. 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 (section 6.2), 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 judgement 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 judgement of the DSMB.

7.3 Procedures for eliciting reports of and for recording and reporting adverse event and intercurrent illnesses

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.

7.4 The type and duration of the follow-up of subjects after adverse events

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 must be followed to resolution and the follow-up evaluations noted in Adverse Event CRF and recorded when the participant has stabilized.

8 STATISTICS

8.1 Description of statistical methods to be employed

Primary outcome

Safety data will be prospectively collected on standardized forms, by a trial nurse blinded to study arm. Standardized toxicity tables, adapted from the National Institute for Allergy and Infectious Diseases (NIAID) Division of Microbiology and Infectious Diseases (DMID) toxicity tables [24] and are reproduced in Appendix 4. 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 titres 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).

Interim analysis

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.

8.2 Number of participants planned to be enrolled

We will enrol 30 participants (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, as described in section 4.2, and 20 patients in Stage 2, in a fully randomized, controlled stage.

To calculate this sample size, we took the J8-specific antibody titre as the continuous immunogenicity design endpoint and used data from a pilot study of the J8-DT vaccine in

8 healthy volunteers. The mean (SD) titre 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 α=0.05 and power=0.8, eight subjects per group would be needed to demonstrate an increase in titres after vaccination compared to commercial vaccine (RABAVERT), by standard sample size calculations for normally distributed data.

8.3 Level of significance to be used

The threshold for statistical significance will be α=0.05.

8.4 Criteria for termination of the trial

The trial will be terminated after enrolment of 30 participants, and completion of all follow-up visits for the last enrolled participant.

The trial will also be continuously monitored for safety by an independent DSMB, who may recommend termination of the trial at an earlier point if unexpected concerns over patient safety arise.

8.5 Procedures for reporting deviations from the original statistical plan

Deviations from the *a priori* statistical analyses described in the protocol will be described and justified in the final report and will be reported as *post hoc* exploratory analyses.

8.6 Selection of participants to be included in analyses

All enrolled participants will be included in the primary *intention-to-treat* analysis. A secondary *per-protocol* analysis may be performed according to the number of doses of vaccine actually administered, in case one or more participants does not receive all three doses of vaccine in the series.

9 DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

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.

10 ETHICS

Declaration of Helsinki

The investigators will ensure that this study is conducted in full conformity with the Declaration of Helsinki (1986), or with the ICH GCP regulations and guidelines, whichever affords greater protection to the trial participants.

Institutional Review Board

The University of Alberta Human Research Ethics Board (HREB) will review this protocol, as well as the consent form and all trial related documents.

No deviation from, or changes of the protocol will be made without prior review and documented approval from the HREB of an amendment,

Informed Consent Process

Written informed consent will be obtained from all trial participants. The consent process shall be initiated at the time of enrolment into the study and shall continue throughout study participation. Participants meeting the eligibility criteria for the study will have the study explained to them by one member of the study team. If the participant provides consent for study participation, he/she will be given the consent forms to sign. A copy of the information sheet will be given to the participant to keep while the signed consent form will be kept in the participant's file.

Subject Confidentiality

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.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Overview

Site initiation, ongoing monitoring and study close out will be performed by the Quality Management in Clinical Research (QMCR) Group within the University of Alberta. http://www.qmcr.ualberta.ca/.

Interim monitoring visits will be conducted to ensure compliance with Good Clinical Practices (GCP), 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 that 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.

Monitoring of ethical standards

In addition to supervision of data entry accuracy and adherence to protocol guidelines, the principal investigators will be 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.

Laboratory analyses, sample collection and storage

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/shipping with the PIs monthly. Discrepancies will be noted, recorded, discussed, and resolved with the PIs.

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

Protocol Deviations

Periodic review of how the protocol is being followed has been discussed above. Minor variations should be captured by QC of the consents and questionnaires, and these will be addressed on a prospective, corrective-action approach with logging in of incident reports. The sum of these problems and corrective actions will be reviewed regularly by the trial manager and the PI to ensure reliability of the study.

Role of the DSMB

A DSMB will provide independent oversight of the trial, with specific attention to participant safety and trial quality. Reports will be submitted to the DSMB chairperson summarizing progress of patient accrual in the trial, and indicators of safety (table of adverse events). The DSMB will meet as a group after Stage 1 to review safety and trial quality. At this point, the DSMB may recommend the trial proceed without modifications, proceed with modifications to the protocol, or be terminated.

12 DATA HANDLING AND RECORDKEEPING

Data Management Responsibilities

The trial nurse will complete the screening form and document baseline health status on the enrolment CRF. The nurse 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, CRF's, 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 Records 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.

13 PUBLICATION POLICY

Following completion of the study, the investigators are expected to publish the results of this research in a scientific journal.

A clear set of guidelines, outlined below, will be given to all potential authors of papers generated by this study. First authors will be expected to have been a major/the major contributor to the work outlined in the paper and to have written the first draft of the paper. As outlined below, all authors must have significantly contributed to the work outlined in the paper. The PI will make the final decisions on authorship for papers resulting from this study.

1. Individuals will be considered for inclusion as authors on work submitted for publications if they have provided one of the following:

- significant contributions affecting the direction, scope, or depth of research
- long-term guidance and development of the project
- creative contributions to the project with clear understanding of its goals
- development of methodologies necessary for timely completion of the project
- data analysis of interpretation vital to conclusions of the project

2. Individuals will not be included as authors for contributions strictly limited to:

- providing laboratory space or use of instrumentation
- providing funding
- services, consulting, or materials provided for a fee or reimbursement
- involvement in patient care or providing patient samples
- routine technical work (as provided by any individual in the lab)
- status as a supervisor, section head, department chairperson

- proofreading or editing of manuscripts
- advice given to solve problems that are narrowly defined or unrelated to the project objective

3. Responsibilities

a) Primary author will:

- Inform all authors and contributors as to how their contributions will be acknowledged.
- Be able to identify the specific contribution of each author.
- Understand the general principles of all work included in a paper.
- Be willing to share openly the data obtained and methodology utilized in the investigation.

b) All authors:

- Be able to defend the methodology and data pertinent to their specific contributions to the project.
- Agree with the general conclusions and interpretations of the paper.

4. Content

- All manuscripts should serve to represent an accurate and complete reflection of the methods utilized and the data obtained in the investigative effort.
- In a publication, all data pertinent to the project should be reported, whether supportive or unsupportive of the thesis or conclusions.
- Except for review articles, publishing the same material in more than 1 paper should be avoided.
- Unnecessary fragmentation of a complete body of work into separate publications should be avoided.
- When ideas, concepts, or text of others are used, appropriate citations should be made.
- Prior work in the field should be referenced appropriately.
- The source of funding should be identified when a work is published.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov [26], which

is sponsored by the National Library of Medicine. We will register this trial on ClinicalTrials.gov.

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APPENDIX 1. PRE-CLINICAL DATA

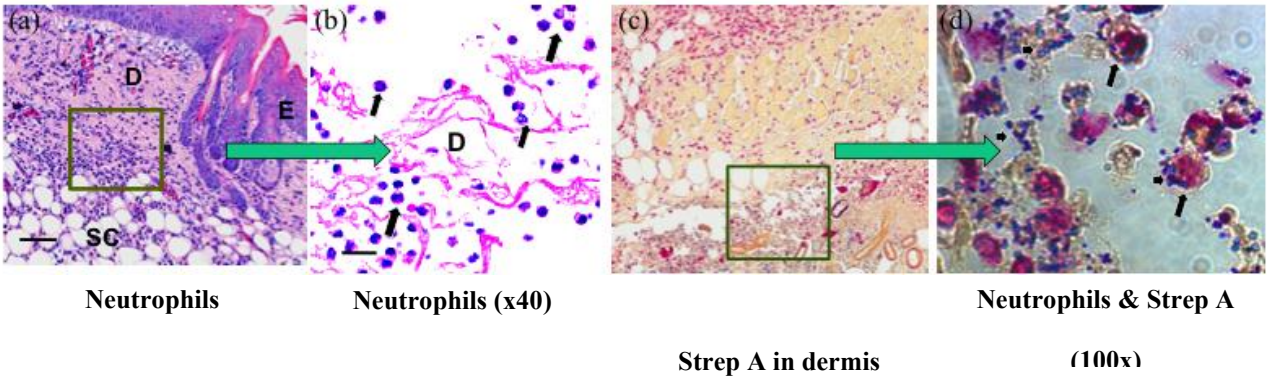

Figure 1: Histopathology of skin lesions infected with Strep A: Photomicrograph of formalin-fixed tissue sections of mouse skin infected with Strep A. Tissue sections are stained with hematoxylin-eosin (a and b) or Gram stain (c and d). Highlighted features are polymorphonuclear cells (PMNs) at the site of inflammation and Strep A trapped within PMNs. E, epidermis; D, dermis; SC, subcutaneous layer.

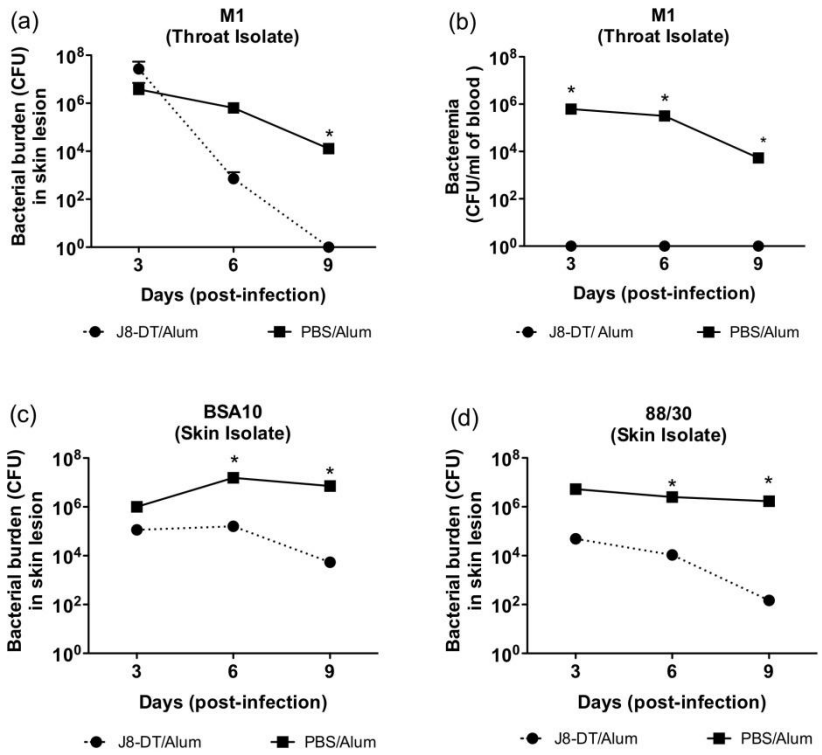

Figure 2: Efficacy of J8-DT/alum immunization on Strep A skin infection: Swiss mice (n=9) were immunized subcutaneously with J8-DT/alum or PBS/alum (days 0, 21 and 28). Four weeks post-immunization, mice were infected with M1 (a and b), 88/30 (c) or BSA10 Strep A (d). Following infection, mice (n=3) were culled on days 3, 6 and 9 and bioburden in skin homogenate and blood was determined. Results are mean +/- SEM. Non-parametric ANOVA with significance ($P<0.05$) between immunised and non-immunised groups marked with an asterisk (*).

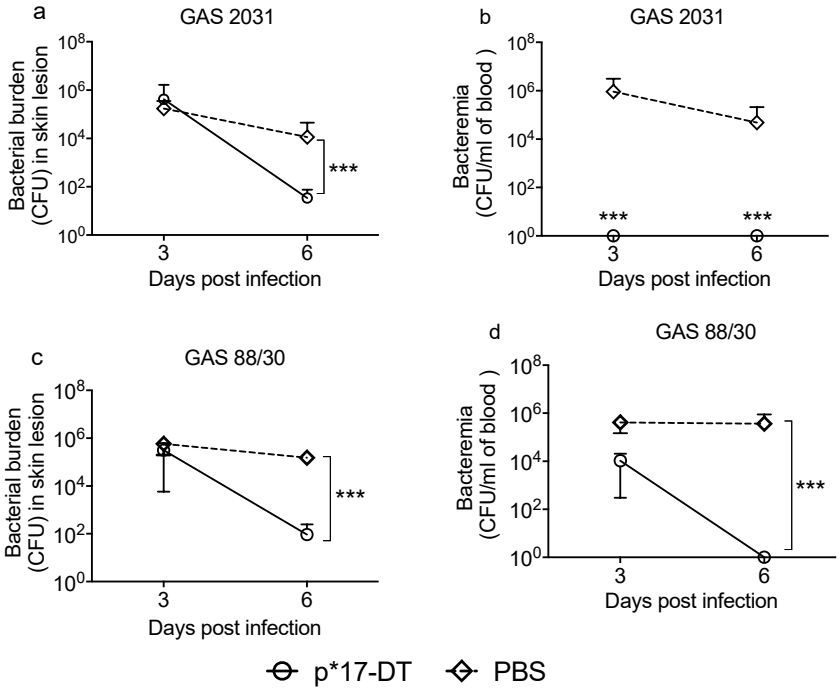

Figure 3: Efficacy of p*17-DT/alum immunization on Strep A skin infection: Balb/c mice (n=10) were immunized subcutaneously with 30µg p*17-DT/alum or PBS/alum (day 0). 21 days post-immunization, mice were infected with Strep A 2031 (a and b), Strep A 88/30 (c and d). Following infection, mice (n=5) were culled on days 3 and 6 and bioburden in skin homogenate and blood was determined. Results are mean +/- SEM. Non-parametric ANOVA with significance ($p<0.001$) between immunised and non-immunised groups marked with an asterisk (***).

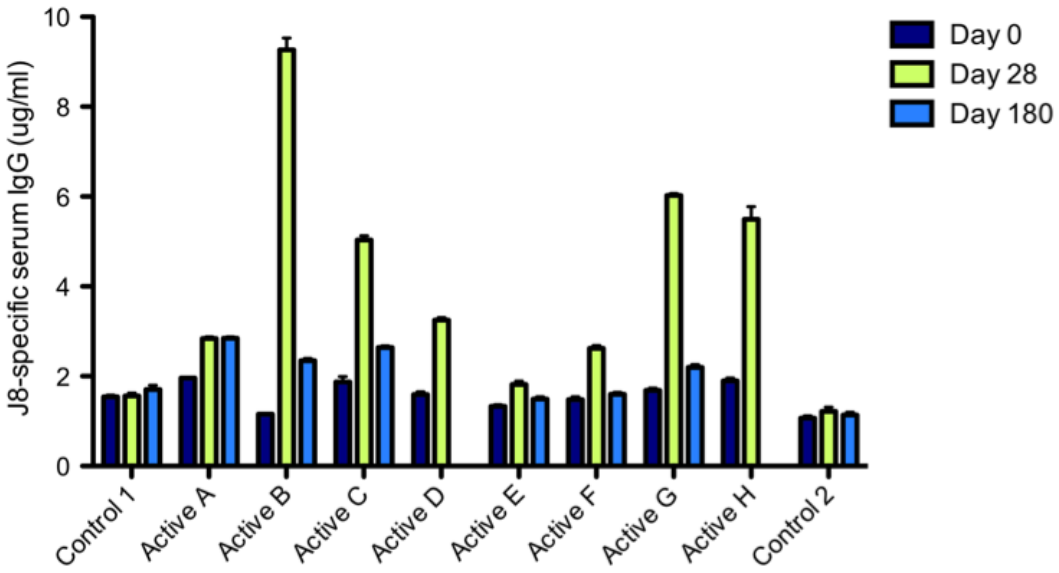

Figure 4: J8-specific serum IgG responses at days 0, 28 and 180 post-primary J8-DT/alum vaccination of volunteers (n=10; 2 controls and 8 active). Subjects Active D and Active H "dropped out" of the study after day 28.

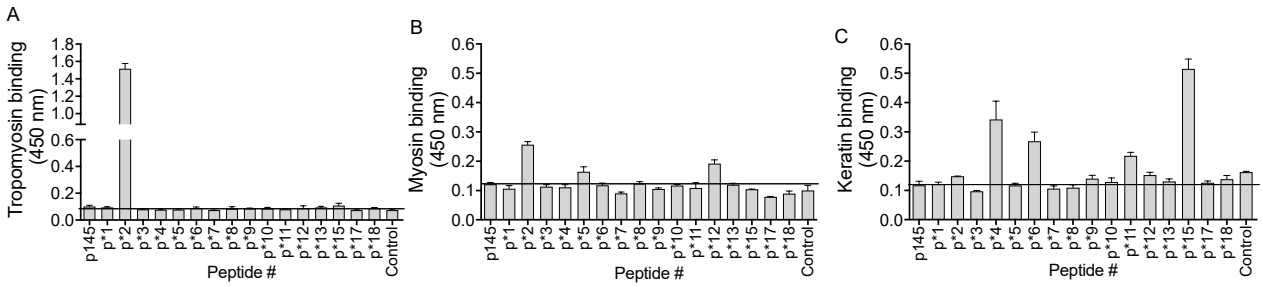

Figure 5: Cross-reactivity of p*17 antisera with human proteins: ELISA was used to determine cross-reactivity of the 18 doubly mutated peptide anti-sera to human derived proteins (a) tropomyosin, (b) myosin and (c) keratin. Mean absorbance (450nm) ± SEM.

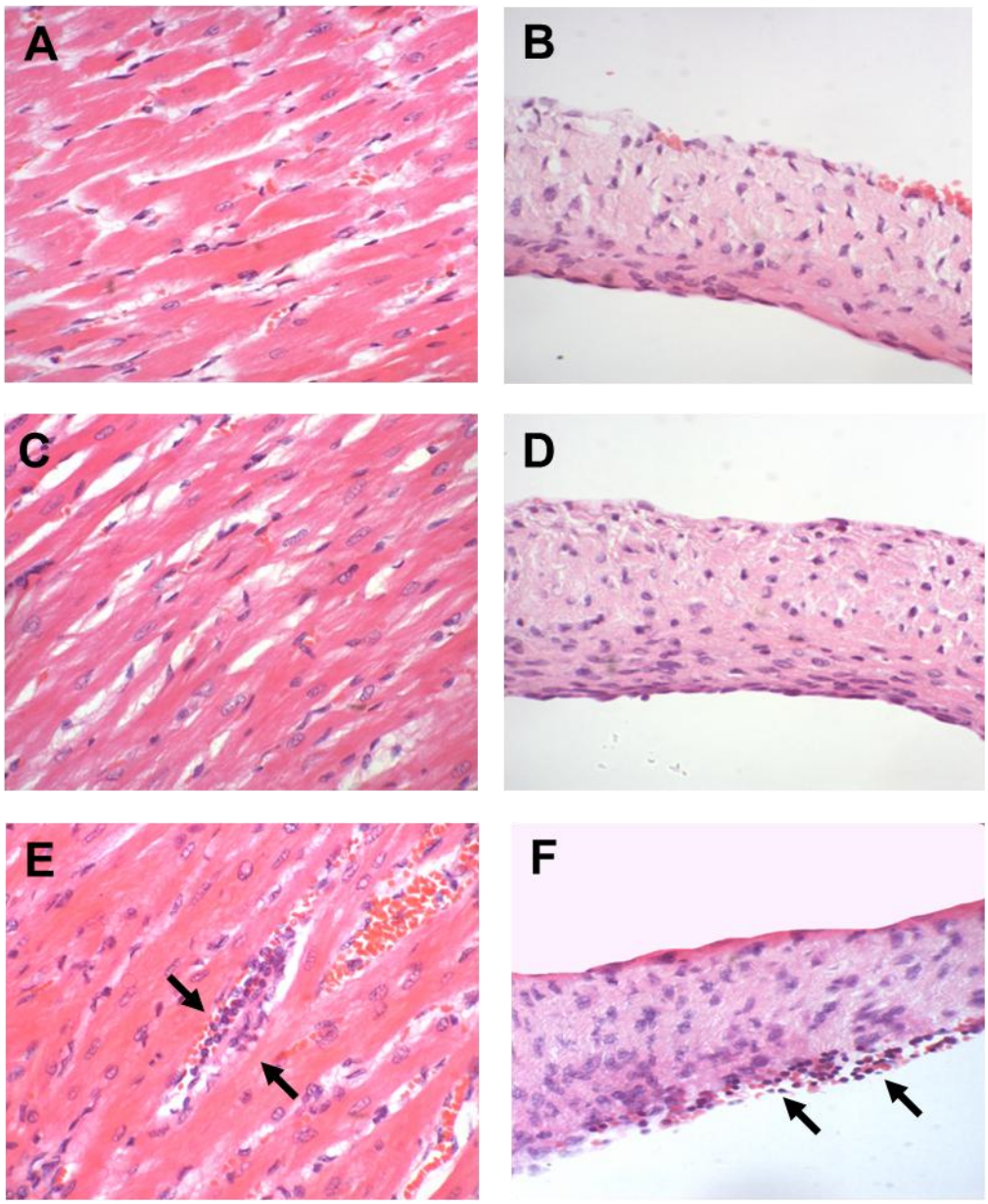

Figure 6: Lewis Rat Model for RHD was also used to assess vaccine safety. Histological features of myocardium (left panel) and heart valves (right panel) from Lewis rats. (A and B) Negative control rats immunized with PBS and adjuvant only showed little or no signs of inflammation. (C and D) Rats immunized with J8-DT had minimal inflammation, comparable to negative control rats. (E and F) rM5-immunized rats showed evidence of mononuclear cell infiltrates (Arrows) in the myocardium and valves. (H&E; original magnifications: A, C and E, x200; B, D and F, x400).rM5 is known to induce rheumatic heart disease in this model.

J8-DT vaccination and skin challenge

Challenge strain:88/30

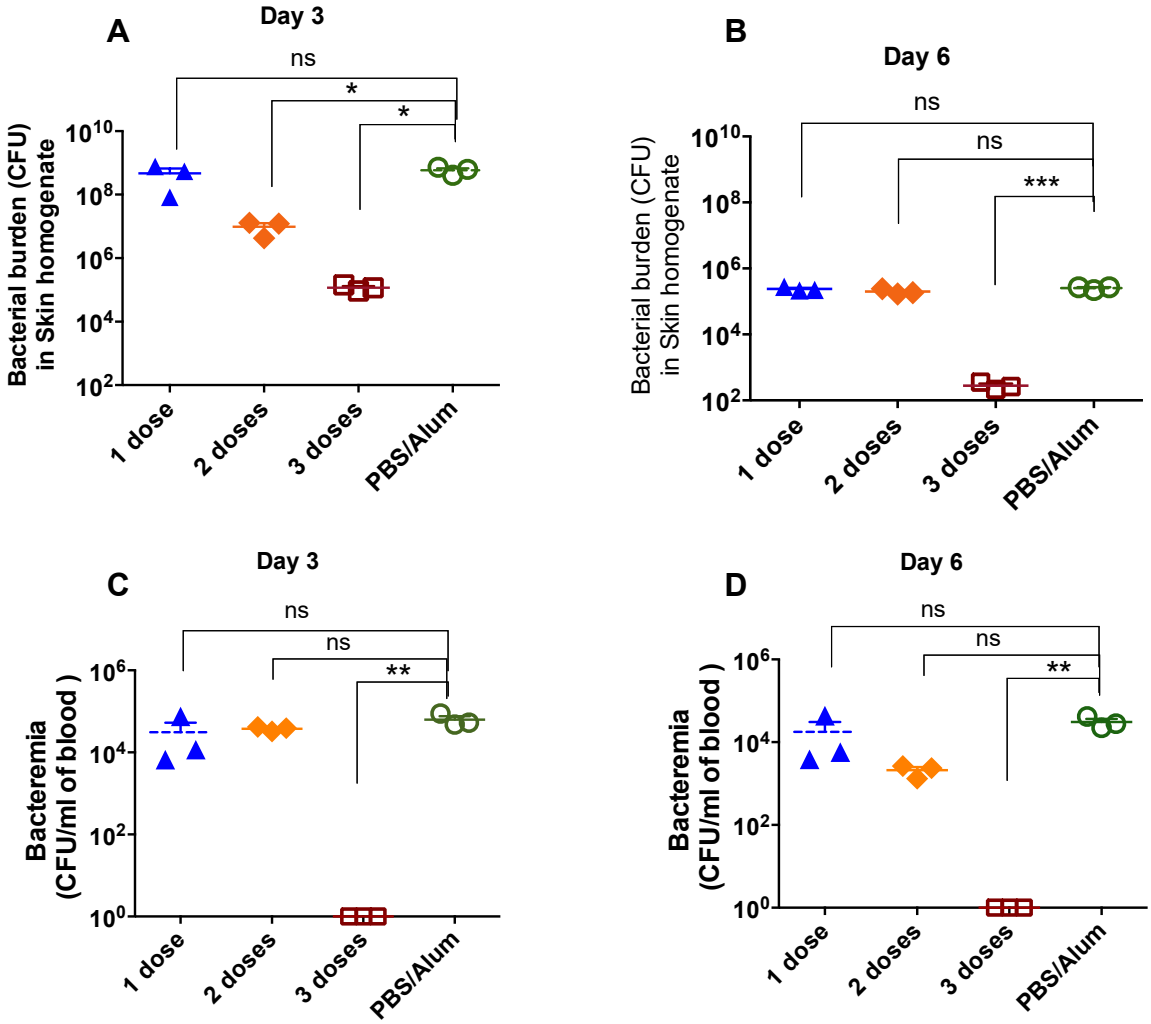

Figure 7: Reduction of Strep A burden in blood (Panels A and B) and skin (Panels C and D) at two time points (3 days, Panels A and C; and 6 days, Panels B and D) after experimental Strep A skin infection was most pronounced in mice who had received 3 prior doses of J8-DT vaccine.

APPENDIX 2. STUDY FLOW CHART

Table 1. Trial Procedures for Stage 1 and Stage 2

	Screening	Enrolment	Follow-up Visits				Unscheduled visit[4]
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-minute 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-DNAase 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 & differential, AST, ALT, glucose, electrolytes, ESR, CRP	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Total cholesterol, HDL	**X**						
Creatinine[3]	**X**	**X**[3]	**X**[3]	**X**[3]	**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**[1]	**X**	**X**	**X**	**X**	**X**	**X**
Echocardiogram	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Memory Aid		**X**	**X**	**X**			
Phone Visit[2]		**X**	**X**	**X**			
Optional Biobanking		**X**	**X**	**X**	**X**	**X**	

*Stage 2 only

1 Blood serum pregnancy test will be performed at the screening visit

2 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.

3 Point-of-care creatinine will be obtained prior to dosing.

4 Unscheduled visits with assessments performed as necessary.

APPENDIX 3. TRIAL DESIGN AND SAFETY CONSIDERATIONS

Table 2. Safety checkpoints in Stage 1, test dosing (n=10).

	Proceed only if safety[1] demonstrated for:
J8-K4S2 (56.25 µg) 1st dose[2]	NA[3]
J8-K4S2 (56.25 µg) 2nd dose	J8-K4S2 (56.25 µg) 1st dose x 5 patients[4]
J8-K4S2 (56.25 µg) 3rd dose	J8-K4S2 (56.25 µg) 2nd dose x 5 patients[4]
p*17-K4S2 (31.25 µg) 1st dose[2]	J8-K4S2 (56.25 µg) 1st dose x 5 patients[4]
p*17-K4S2 (31.25 µg) 2nd dose	J8-K4S2 (56.25 µg) 2nd dose x 5 patients[4] & p*17-K4S2 (31.25 µg) 1st dose x 5 patients[4]
p*17-K4S2 (31.25 µg) 3rd dose	J8-K4S2 (56.25 µg) 3rd dose x 5 patients[4] & p*17-K4S2 (31.25 µg) 2nd dose x 5 patients[4]

[1]Safety: absence of severe adverse event, grade 4 adverse event (based on standardized toxicity tables), no valvulitis on echocardiogram, and no episode of acute rheumatic fever
[2]One patient will receive test, dose, wait two days, assess safety, and administer test dose to second patient after this time, if safety has been established, and so on, until 5 participants have safety received test doses.
[3]J8 has been previously shown to be safe at 50 µg, the purpose of this test dose is to verify the safety of the K4S2 component.
[4]For each set of doses, all 5 patients in the group would need to have received the dose, and safety established (see Footnote 1), before proceeding to the subsequent set.

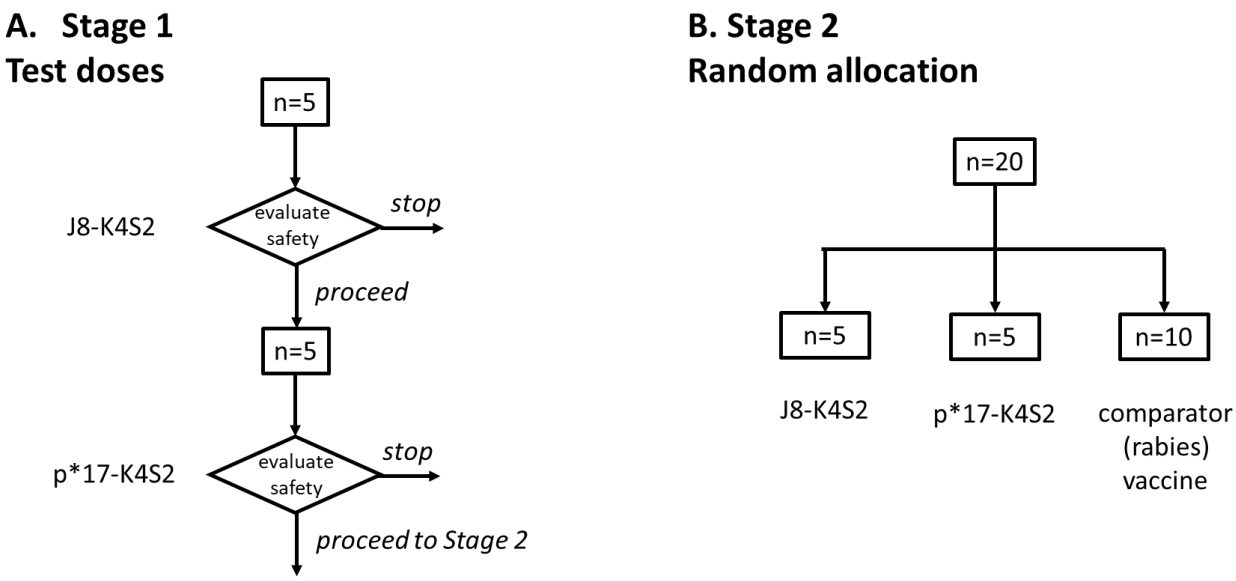

Figure 8. Trial profile. The trial involves 2 stages. Stage 1 (Panel A) involves test doses of the J8-K4S2 and p*17-K4S2 vaccines. Using a small number of participants (5+5), we will assess the immediate safety. We will start with J8-K4S2 (one patient at a time, separated by at least two days with safety assessment prior to immunizing another participant), then proceed to p*17-K4S2 (one patient at a time). Stage 2 (Panel B) will randomize an additional 20 patients to J8-K4S2 (n=5), p*17-K4S2 (n=5) or comparator (rabies) vaccine (n=10). At the end of the trial, data will be pooled (Stage 1 and 2), such that there will be 10 patients in each experimental arm and 10 patients in the comparator vaccine arm.

APPENDIX 4. STANDARDIZED TOXICITY TABLES

HEMATOLOGY				
	Grade 1 (Mild)	**Grade 2 (Moderate)**	**Grade 3 (Severe)**	**Grade 4 (Potentially life threatening)**
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	6.5 - 7.9 gm/dL	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/mm3	750-999/mm3	500-749/mm3	<500/mm3
Platelets	75,000-99,999/mm3	50,000-74,999/mm3	20,000-49,999/mm3	<20,000/mm3
WBCs	11,000-13,000/mm3	13,000- 15,000 /mm3	15,000-30,000/mm3	>30,000 or <1,000 /mm3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	----------
CHEMISTRIES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hyponatremia	130 to<135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus, or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (non-fasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required
ENZYMES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
URINALYSIS				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion
CARDIOVASCULAR				

	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac Rhythm	asymptomatic, no Rx required	asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrhythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused
RESPIRATORY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Cough	transient- no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV1 of peak flow	requires treatment; normalizes with bronchodilator; FEV1 50% - 70% (of peak flow)	no normalization with bronchodilator; FEV1 25% - 50% of peak flow; or retractions present	cyanosis: FEV1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring hospitalization
GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	constipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose	moderate or persistent; 5-7 loose	>7 loose stools/day or	hypotensive shock or physiologic

	stools/day or mild diarrhea last < 1 week	stools/day or diarrhea lasting >1 week	bloody diarrhea; or orthostatic hypotension or electrolyte imbalance or >2L IV fluids required	consequences requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids
NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	subjective weakness no objective symptoms/ signs	mild objective signs/symptoms, no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures
SKIN				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Mucocutaneous	erythema; pruritus	diffuse, maculopapular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	**< 15mm**	15-30 mm	>30mm	
Erythema	**< 15mm**	15-30 mm	>30mm	
Edema	**< 15mm**	15-30 mm	>30mm	
Rash at Injection Site	**< 15mm**	15-30 mm	>30mm	

Pruritus	slight itching at injection site	moderate itching at injection extremity	itching	
SYSTEMIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	Severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25- 50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

Additionally, table below* describes a severity grading system for any clinical adverse event not identified in appendix 4, standardized toxicity table.

Grade 1 (mild)	**Grade 2** (moderate)	**Grade 3** (severe)	**Grade 4** (potentially life-threatening)
Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

*Adapted from the NIH Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events.

APPENDIX 5. CALCULATION OF FRAMINGHAM CARDIOVASCULAR RISK SCORE

Women
Age: 20–34 years: Minus 7 points. 35–39 years: Minus 3 points. 40–44 years: 0 points. 45–49 years: 3 points. 50–54 years: 6 points. 55–59 years: 8 points. 60–64 years: 10 points. 65–69 years: 12 points. 70–74 years: 14 points. 75–79 years: 16 points.

Total cholesterol, mg/dL*: Age 20–39 years: Under 160: 0 points. 160-199: 4 points. 200-239: 8 points. 240-279: 11 points. 280 or higher: 13 points. • Age 40–49 years: Under 160: 0 points. 160-199: 3 points. 200-239: 6 points. 240-279: 8 points. 280 or higher: 10 points. • Age 50–59 years: Under 160: 0 points. 160-199: 2 points. 200-239: 4 points. 240-279: 5 points. 280 or higher: 7 points. • Age 60–69 years: Under 160: 0 points. 160-199: 1 point. 200-239: 2 points. 240-279: 3 points. 280 or higher: 4 points. • Age 70–79 years: Under 160: 0 points. 160-199: 1 point. 200-239: 1 point. 240-279: 2 points. 280 or higher: 2 points.

If cigarette smoker: Age 20–39 years: 9 points. • Age 40–49 years: 7 points. • Age 50–59 years: 4 points. • Age 60–69 years: 2 points. • Age 70–79 years: 1 point.

All non smokers: 0 points.

HDL cholesterol, mg/dL*: 60 or higher: Minus 1 point. 50-59: 0 points. 40-49: 1 point. Under 40: 2 points.

Systolic blood pressure, mm Hg: Untreated: Under 120: 0 points. 120-129: 1 point. 130-139: 2 points. 140-159: 3 points. 160 or higher: 4 points. • Treated: Under 120: 0 points. 120-129: 3 points. 130-139: 4 points. 140-159: 5 points. 160 or higher: 6 points.

10-year risk in %: Points total: Under 9 points: <1%. 9-12 points: 1%. 13-14 points: 2%. 15 points: 3%. 16 points: 4%. 17 points: 5%. 18 points: 6%. 19 points: 8%. 20 points: 11%. 21=14%, 22=17%, 23=22%, 24=27%, >25= Over 30%

Men
Age: 20–34 years: Minus 9 points. 35–39 years: Minus 4 points. 40–44 years: 0 points. 45–49 years: 3 points. 50–54 years: 6 points. 55–59 years: 8 points. 60–64 years: 10 points. 65–69 years: 11 points. 70–74 years: 12 points. 75–79 years: 13 points.

Total cholesterol, mg/dL*: Age 20–39 years: Under 160: 0 points. 160-199: 4 points. 200-239: 7 points. 240-279: 9 points. 280 or higher: 11 points. • Age 40–49 years: Under 160: 0 points. 160-199: 3 points. 200-239: 5 points. 240-279: 6 points. 280 or higher: 8 points. • Age 50–59 years: Under 160: 0 points. 160-199: 2 points. 200-239: 3 points. 240-279: 4 points. 280 or higher: 5 points. • Age 60–69 years: Under 160: 0 points. 160-199: 1 point. 200-239: 1 point. 240-279: 2 points. 280 or higher: 3 points. • Age 70–79 years: Under 160: 0 points. 160-199: 0 points. 200-239: 0 points. 240-279: 1 point. 280 or higher: 1 point.

If cigarette smoker: Age 20–39 years: 8 points. • Age 40–49 years: 5 points. • Age 50–59 years: 3 points. • Age 60–69 years: 1 point. • Age 70–79 years: 1 point.

All non smokers: 0 points.

HDL cholesterol, mg/dL*: 60 or higher: Minus 1 point. 50-59: 0 points. 40-49: 1 point. Under 40: 2 points.

Systolic blood pressure, mm Hg: Untreated: Under 120: 0 points. 120-129: 0 points. 130-139: 1 point. 140-159: 1 point. 160 or higher: 2 points. • Treated: Under 120: 0 points. 120-129: 1 point. 130-139: 2 points. 140-159: 2 points. 160 or higher: 3 points.

10-year risk in %: Points total: 0 point: <1%. 1-4 points: 1%. 5-6 points: 2%. 7 points: 3%. 8 points: 4%. 9 points: 5%. 10 points: 6%. 11 points: 8%. 12 points: 10%. 13 points: 12%. 14 points: 16%. 15 points: 20%. 16 points: 25%. 17 points or more: Over 30%.

*Note that Alberta Health Services reports high-density lipoprotein (HDL) and total cholesterol (TC) in units of mmol/L. To convert cholesterol units of TC and HDL from mmol/L to mg/dL, multiply by 38.67.