Statistical Analysis Plan

Title:

A phase I, double-blind, parallel, randomised and placebo-controlled trial investigating the safety and immunogenicity of a chlamydia vaccine, CTH522, in healthy adults

A Phase I Trial

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Table of Contents

1 List of Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
CI	Confidence Interval
CRF	Case Report Form
CV	Coefficient of Variation
DBL	Data Base Lock
FAS	Full Analysis Set
ID	Intradermal
IgG	Immunoglobulin G
IM	Intramuscular
IMP	Investigational Medical Product
MedDRA	Medical Dictionary for Regulatory Activities
PP	Per Protocol
PT	Preferred Term
SAE	Serious Adverse Event
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TO	Topical ocular

2 Introduction

The statistical analysis plan for the CHLM-02 is based on the final protocol version 5.1 dated 14DEC2020.

The SAP describes in detail the analyses to be conducted and highlights any deviations from the analysis described in the protocol (see section 8). Deviations from methods described in this SAP, if any, will be specified in the clinical trial report.

Before releasing data for final analysis, one or more data review and classification meeting will be held to review protocol deviations to classify subjects with respect to analysis populations. The product of the classification meetings will be a detailed description of the analysis populations, and the number and nature of unresolved data queries will also be reported.

The analysis is performed based on:

- The clinical database, which includes the electronic Case Report Forms (CRF)
- List of protocol deviations
- Analysis populations documented in the Data Base Lock minutes

Reporting of the trial will be after the date of last subject last visit.

3 Trial Characteristics

3.1 Trial Objectives

3.1.1 Primary Objective

- To evaluate the safety of CTH522-CAF01 administered Intramuscular (IM) in different doses
- To evaluate the safety of non-adjuvanted CTH522 administered Topical ocular (TO) or Intradermal (ID) simultaneously with CTH522-CAF01 IM
- To evaluate the safety of CTH522-CAF09b administered IM

3.1.2 Secondary Objectives

- To evaluate the serum Immunoglobulin G (IgG) antibody responses obtained after IM administration of CTH522-CAF01 in different doses
- To evaluate the serum IgG antibody responses obtained after TO or ID administration of non-adjuvanted CTH522 simultaneously with CTH522-CAF01 IM
- To evaluate the serum IgG antibody responses obtained after IM administration of CTH522-CAF09b
- To evaluate the serum IgG antibody response obtained after TO administration of non-adjuvanted CTH522 on Day 140

3.1.3 Exploratory Objectives

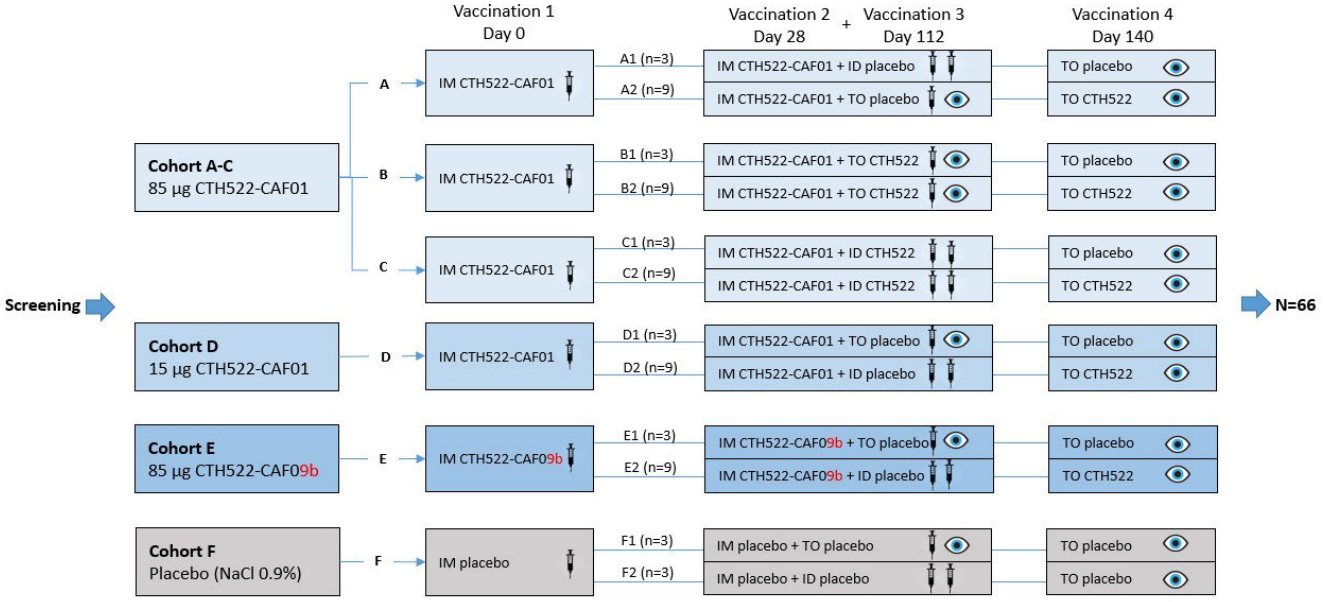
- To evaluate the systemic and mucosal immunogenicity after vaccination with CTH522 with different adjuvants and administration routes

3.2 Trial Design

The trial is a phase I, double-blind, parallel, randomised, and placebo-controlled trial of the chlamydia vaccine, CTH522, in healthy adults. The trial will be conducted at Imperial College Healthcare NHS Trust, London, in the UK.

It is planned to randomly assign 66 subjects into 12 treatment groups in six Cohorts. Cohorts A-D investigate CTH522-CAF01 administered IM in two doses (85 µg and 15 µg). Cohort E investigates CTH522-CAF09b administered IM in one dose (85 µg). Cohort F is the placebo group (Figure 1). The enrolled subjects will complete 12 trial visits.

Figure 1: Trial design



IM=intramuscular injection with CTH522-CAF01 or CTH522-09b 85 µg or 15 µg; ID=intradermal injection with non-adjuvanted CTH522 24 µg; TO=topical ocular with non-adjuvanted CTH522 12 µg in each eye

Table 1: Flow chart

Trial visit (V)	1	2	3	4	5	6	7	8	9	10	11	12
Trial day	-	0	14	28	42	56	112	126	140	143	154	238
Windows (days)	-90 to -1	0	V2 + 12-16	V2 + 26-30	V4 + 12-16	V4 + 26-30	V4 + 77-91	V7 + 12-16	V7 + 26-30	V9 + 2-4	V9 + 12-16	V9 + 91-105
Informed consent	X											
Med. hist+demographics	X											
In-/exclusion criteria	X	X										
Blood for hep B and C, HIV, syphilis	X											
PCR for *C. Trachoma* and gonorrhoea infection[1]	X											X
Urinary pregnancy test	X	X		X			X		X			X
Physical examination	X											X
Eye exam including retina*	X*				X			X			X	
Eye exam**	X	X		X	X	X	X	X	X	X	X	X
Record weight/height	X											
Vital signs[2]	X	X	X	X	X		X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X
Randomisation		X										
Vaccination and route		1 IM		2 IM ID TO			3 IM ID TO		4 TO			
Issue diary card		X		X			X		X			
Review diary card			X		X			X		X	X	
Symptom-directed PE		X	X	X	X	X	X	X	X	X	X	X
Record adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Safety lab[3]	X[4]	X[5]	X[5]	X[5]	X[5]		X[5]	X[5]	X[5]		X[5]	X[5]
Serum IgG ELISA[6]		X	X	X	X	X	X	X	X		X	X
Serum IgA ELISA+anti-CTH522 IgG subtypes + neutralising ab[6]		X	X	X	X	X	X	X	X		X	X
Ocular anti-CTH522 IgG + IgA ELISA (strip)		X		X	X	X	X	X	X	X	X	X
T- and B-cell Elispot frozen PBMCs[7]		X	X	X	X	X	X	X	X		X	X
ICS flow frozen PBMCs[7]		X	X	X	X	X		X	X		X	X
Transcriptional analysis frozen PBMCs[8]		X						X			X	
End-of-trial												X

ID=intradermal; IM=intramuscular; PE=physical examination; TO=topical ocular; V=visit

*A biomicroscopy (e.g. via slit lamp examination), examining all areas of the eye including the retina which can be performed by a specialist at an eye clinic. If this procedure cannot be performed at V1, it is acceptable at V2 as long as it is performed prior to the first vaccination.

**Eye examination not involving fundoscopy or any other examination modality that necessitates unacceptable prolonged closeness between examiner and participant or where the examination cannot be performed using standard personal protective equipment for examiner and participant.

[1]single urine PCR tests for *C. Trachoma* and gonorrhoea; [2]blood pressure, heart rate and oral temperature; taken pre-vaccination when scheduled at vaccination visits (V2, V4, V7 and V9); [3]Safety lab: renal urea and electrolytes (sodium, potassium, chloride, urea, and creatinine); liver function tests (alanine aminotransferase, alkaline phosphatase, total bilirubin, total protein, albumin); full blood count (WBC, lymphocytes, monocytes, eosinophils, neutrophils, basophils, RBC, HGB, ´HCT, and platelets): [4]total blood for safety: approximately 13 ml; [5]total blood for safety: approximately 8 ml; [6]amount of blood: approximately 1 x 10 ml; [7]amount of blood in heparin tubes: 5 x 6 ml; [8]amount of blood in heparin tubes: 2 x 6 ml

3.2.1 Treatment labels

The cohort labels will be used as treatment labels in tables, figures, and listings as described in Table 2. Cohorts who had the same treatment at first, second and/or third vaccination will when relevant be grouped at timepoints where the treatments received were similar, and will be labelled accordingly:

Table 2: Treatment labels

Day and treatment	Cohort	Label
Day 0		
85 µg IM CTH522-CAF01	A1, A2, B1, B2, C1, C2	Cohort A-C CTH-01-all
15 µg IM CTH522-CAF01	D1, D2	Cohort D CTH-01-low
85 µg IM CTH522-CAF09b	E1, E2	Cohort E CTH-09
IM Placebo	F1, F2	Cohort F Placebo
Day 28 and 112		
85 µg IM CTH522-CAF01 + ID Placebo	A1	Cohort A1 CTH-01+ID-PCB
85 µg IM CTH522-CAF01 + TO Placebo	A2	Cohort A2 CTH-01+TO-PCB
85 µg IM CTH522-CAF01 + TO CTH522	B1, B2	Cohort B CTH-01+TO
85 µg IM CTH522-CAF01 + ID CTH522	C1, C2	Cohort C CTH-01+ID
15 µg IM CTH522-CAF01 + TO Placebo	D1	Cohort D1 CTH-01-low+TO-PCB
15 µg IM CTH522-CAF01 + ID Placebo	D2	Cohort D2 CTH-01-low+ID-PCB
85 µg IM CTH522-CAF09b + TO Placebo	E1	Cohort E1 CTH-09+TO-PCB
85 µg IM CTH522-CAF09b + ID Placebo	E2	Cohort E2 CTH-09+ID-PCB
IM Placebo + TO Placebo	F1	Cohort F1 Placebo+TO-PBO
IM Placebo + ID Placebo	F2	Cohort F2

		Placebo+ID-PCB
Day 140		
85 µg IM CTH522-CAF01 + ID Placebo + TO Placebo	A1	Cohort A1 CTH-01+ID-PCB+TO-PCB
85 µg IM CTH522-CAF01 + TO Placebo + TO CTH522	A2	Cohort A2 CTH-01+TO-PCB+TO
85 µg IM CTH522-CAF01 + TO CTH522 + TO Placebo	B1	Cohort B1 CTH-01+TO+TO-PCB
85 µg IM CTH522-CAF01 + TO CTH522	B2	Cohort B2 CTH-01+TO
85 µg IM CTH522-CAF01 + ID CTH522 + TO placebo	C1	Cohort C1 CTH-01+ID+TO-PCB
85 µg IM CTH522-CAF01 + ID CTH522 + TO CTH522	C2	Cohort C2 CTH-01+ID+TO
15 µg IM CTH522-CAF01 + TO Placebo	D1	Cohort D1 CTH-01-low+TO-PCB
15 µg IM CTH522-CAF01 + ID Placebo + TO CTH522	D2	Cohort D2 CTH-01-low+ID-PCB+TO
85 µg IM CTH522-CAF09b + TO Placebo	E1	Cohort E1 CTH-09+TO-PCB
85 µg IM CTH522-CAF09b + ID Placebo + TO CTH522	E2	Cohort E2 CTH-09+ID-PCB+TO
IM Placebo + TO Placebo	F1	Cohort F1 Placebo+TO-PCB
IM Placebo + ID Placebo + TO Placebo	F2	Cohort F2 Placebo+ID-PCB+TO-PCB

The full treatment will be explained in footnotes. In the above CTH-01 is an abbreviation for CTH522-CAF01, and CTH-09 for CTH522-CAF09b.

3.3 Subjects included

It is planned to screen approximately 110 subjects to randomise 66 subjects. The subjects should be healthy male and female volunteers aged 18–45 years.

No sample size calculations have been made. The sample size is considered adequate for review of the safety profile of the described interventions for a phase I trial.

Number of randomised subjects to each treatment group is presented in Figure 1 above.

4 Analysis Populations

The following 3 analysis sets are defined:

- Safety analysis set (SAF): The SAF consist of all subjects randomised and exposed to the investigational medical products (IMPs), including subjects who are withdrawn after exposure to the IMP.
- Full analysis set (FAS): FAS will include subjects with observed data for all randomised subjects.
- Per Protocol (PP) analysis set: PP analysis set will include subjects who completed the trial without deviations judged to influence the primary endpoints. Please refer to section 4.1 for details.

Since all randomised subjects have observed data, FAS will consist of all randomised subjects.

The basis for safety analysis is the SAF. The analysis of all safety endpoints will be based on the SAF, and the subjects will be evaluated according to actual treatment.

The analysis sets will be defined at the data release meeting, which will take place prior to unblinding of the trial.

4.1 Major protocol deviations

Before data base lock (DBL) all protocol deviations will be evaluated. Protocol deviations having a major impact on the co-primary endpoints will be identified and lead to exclusion from PP. The decisions will be documented.

5 Planned Statistical Methods

If nothing else is stated, all tables, figures and listings will be presented by treatment group (i.e., A1, A2, B1, B2, …, F2), and will be reported by actual received treatment for the SAF. A subject who receives more or less of the treatment than intended, will be presented in the cohort they belong to. If any subjects receive incorrect treatment, they will be presented separately.

5.1 Statistical Considerations

Baseline is defined as the last assessment with available data prior to the first administration of trial medication. Day 0 is the day of first administration with IMP. Assessments from scheduled visits will be presented when available, otherwise the first unscheduled visit succeeding the scheduled visit in question is used.

Categorical data will be summarized by treatment group, using number and percentages of subjects. For calculation of percentages the denominator will either be the number of subjects in the analysis set or the number of subjects in the analysis set with non-missing values. Continuous data will be presented using the number of subjects (n), mean, standard deviation (SD), median, minimum, and maximum if nothing else is stated. Both the absolute values and the change from baseline will be presented.

Descriptive statistics for all endpoints will be presented by treatment group and visit (if applicable) using observed cases, i.e., no imputation of missing data will be performed.

All statistical tests will be two-sided and performed on a 5 % significance level. Estimated treatment differences and 95 % confidence intervals (CI) will be presented together with the corresponding p-value.

5.2 Subject Disposition

An overall summary table of the subject disposition will be prepared with number of subjects in the following categories and sub-categories:

- Screened subjects
  - Screening failures
  - Not assigned
  - Randomised
- Analysis sets
    - SAF
    - FAS
    - PP population
- Completers
- Withdrawals with reason:
    - Adverse event
    - Screen failure
    - Withdrawal by subject
    - Lost to follow-up
    - Pregnancy
    - Death
    - Protocol violation
    - Investigator decision
    - Sponsor request
    - Other

All information will be listed.

5.3 Protocol Deviations

Protocol deviations consist of the types

- Inclusion/Exclusion criteria
- Prohibited Medication
- Visit out of window
- Lab
- Other

and will be categorised before unblinding of the trial as major or minor, and those leading to exclusion from PP population will be identified and documented.

Protocol deviations will be summarised for all randomised subjects by category and type. Covid-19 related deviations are identified before DBL and will be summarised by category.

All deviations will be listed.

5.4 Baseline Characteristics and Demographics

Baseline characteristics and demographics consist of age, gender, race, weight, height, and BMI.

Demographics will be summarized using descriptive statistics. Age and gender will in addition be summarised for FAS and PP.

All information will be listed.

5.5 Medical History

Medical history will be summarised by system organ class (SOC) and preferred term (PT).

All information will be listed.

6 Exposure and Other Dosing Information

6.1 Exposure

Number of vaccinations according to protocol will be summarised by type

- Intramuscular (IM)
- Intradermal (ID)
- Topical ocular (TO)

and by content

- CTH522
- Placebo

Treatment exposure is assessed by time from first vaccination to end of trial and will be summarised in days.

All data will be listed.

6.2 Prior and concomitant medication

Medications stopped before first treatment with IMP is entitled prior medication, and other medications concomitant medication. The medications are coded using WHO Drug DDE – Sep 2019 and both will be summarised by ATC level 3 and drug name and will also listed by subject.

7 Statistical Methodology for Primary and Secondary Endpoints

7.1 Analysis and Presentation of the co-Primary Safety Endpoints

The co-primary endpoints are:

- Solicited local injection site reactions after ID and/or IM administration of the vaccines: erythema, pruritus, pain, tenderness, swelling, and warmth occurring up to and including 14 days after any IMP administration
- Solicited local reactions after TO administration of the vaccine: watering eyes, swelling of eyelid, eye redness, and eye discomfort occurring up to and including 14 days after any IMP administration
- Solicited systemic reactions after IM and/or ID administration of the vaccines: oral temperature > 38.3°C, chills, myalgia, and rash occurring up to and including 14 days after any IMP administration
- Any other adverse events (AEs)

These are collected in the subjects' diaries.

The primary analyses will be carried out for the SAF.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 22.1 - Sep 2019. AEs will be regarded as treatment emergent AEs (TEAEs) if they occur after administration of randomised treatment.

Only TEAEs will be summarised. The summaries will include number of events, number of subjects, and proportion of subjects reporting these events.

7.1.1 Solicited injection site reactions

Solicited injection site reactions after IM and/or ID administration are defined as AEs with the following PTs:

- Injection site erythema
- Injection site pruritus
- Injection site pain (covers both the lower level terms *injection site tenderness* and *injection site pain*)
- Injection site swelling
- Injection site warmth

occurring in the period after and up to and including 14 days after Day 0, Day 28, and Day 112 vaccination.

The number and percentage of subjects and the number of events will be presented by PT:

- At Day 0 for the four treatment regimens present

  - Cohort A-C
  - Cohort D
  - Cohort E
  - Cohort F

- At Day 28 and Day 112 for the 10 treatment regimens present
  - Cohort A1
  - Cohort A2
  - Cohort B
  - Cohort C
  - Cohort D1
  - Cohort D2
  - Cohort E1
  - Cohort E2
  - Cohort F1
  - Cohort F2

The number and percentage of subjects with events occurring in the period after and up to and including 14 days after the Day 0 vaccination and the corresponding 95% CI will be presented together with a pairwise comparison of number of subjects with events using Fishers exact test for the following:

- Cohort A-C vs Cohort D
- Cohort A-C vs Cohort E
- Cohort A-C vs Cohort F

All information will be listed.

7.1.2 Solicited ocular reactions

Solicited local reactions after TO administration are defined as AEs with the following PTs:

- Lacrimation increased
- Swelling of eyelid
- Ocular hyperaemia
- Ocular discomfort

occurring in the period after and up to and including 14 days after Day 28, Day 112, and Day 140 vaccinations.

The number and percentage of subjects and the number of events will be presented by PT:

- At Day 28 and Day 112 for the five TO treatment regimens
  - Cohort A2
  - Cohort B
  - Cohort D1

  - Cohort E1
  - Cohort F1

- At Day 140 for the 11 TO treatment regimens present, where the placebo cohort (F) will be regarded as one
  - Cohort A1
  - Cohort A2
  - Cohort B1
  - Cohort B2
  - Cohort C1
  - Cohort C2
  - Cohort D1
  - Cohort D2
  - Cohort E1
  - Cohort E2
  - Cohort F

The number and percentage of subjects with events occurring in the period after and up to and including 14 days after the Day 28 and the Day 112 vaccinations and the corresponding 95% CI will be presented by visit together with a pairwise comparison of number of subjects with events using Fishers exact test for the following:

- Cohort B vs Cohort A2
- Cohort B vs Cohort F1

All information will be listed.

7.1.3 Solicited systemic reactions

Solicited systemic reactions after IM and/or ID administration are defined as AEs with the following PTs:

- Pyrexia
- Chills
- Myalgia
- Rash

occurring in the period after and up to and including 14 days after the Day 0, Day 28, and Day 112.

Pyrexia should be used as PT for temperatures above 38.3°C only, which is not as in the FDA guidelines presented in the protocol Appendix 1 stating temperatures above 38.0°C. For increased temperatures at or below 38.3°C, the PT *Body temperature increased* is used and thus not included as events. If the temperature is unknown, *Pyrexia* will be used, and the event included.

The number and percentage of subjects and the number of events will be presented by PT:

- At Day 0 for the four treatment regimens present
  - Cohort A-C
  - Cohort D
  - Cohort E
  - Cohort F

- At Day 28 and Day 112 for the 10 treatment regimens present
  - Cohort A1
  - Cohort A2
  - Cohort B
  - Cohort C
  - Cohort D1
  - Cohort D2
  - Cohort E1
  - Cohort E2
  - Cohort F1
  - Cohort F2

The number and percentage of subjects with events occurring in the period after and up to and including 14 days after the Day 0 vaccination and the corresponding 95% CI will be presented together with a pairwise comparison of number of subjects with events using Fishers exact test for the following:

- Cohort A-C vs Cohort D
- Cohort A-C vs Cohort E
- Cohort A-C vs Cohort F

All information will be listed.

7.1.4 Any other AEs

Any other AEs are all TEAEs not included in the above three sections.

The number and percentage of subjects and the number of events will be presented by system organ class (SOC) and preferred term (PT) for the following timespans:

- From Day 0 vaccination to before Day 28 vaccination for the four treatment regimens present
  - Cohort A-C
  - Cohort D
  - Cohort E
  - Cohort F

- From Day 28 vaccination to before Day 112 vaccination and
- From Day 112 vaccination to before Day 140 vaccination
  The immediate above two tables will include the nine treatment regimens present, where the placebo cohort (F) will be regarded as one

- Cohort A1
- Cohort A2
- Cohort B
- Cohort C
- Cohort D1
- Cohort D2
- Cohort E1
- Cohort E2
- Cohort F

- From Day 140 vaccination to end of trial for the 11 treatment regimens present, where the placebo cohort (F) will be regarded as one
  - Cohort A1
  - Cohort A2
  - Cohort B1
  - Cohort B2
  - Cohort C1
  - Cohort C2
  - Cohort D1
  - Cohort D2
  - Cohort E1
  - Cohort E2
  - Cohort F

7.2 Analysis and Presentation of Other Safety Endpoints

Other safety endpoints are

- AEs
- Vital signs
- Safety laboratory data

7.2.1 Adverse Events

The summaries will include number of events, number of subjects, and proportion of subjects reporting these events.

Certain, probable, or possible related AEs are defined as Adverse Drug Reactions (ADRs).

An overall summary table will include summary of TEAEs, TESAEs, Fatal AEs, ADRs, AEs leading to drug withdrawal, drug interruption, or leading to withdrawal from trial, AEs by severity, AEs by relationship, AEs by outcome, and ADRs by severity.

A summary by SOC and PT will be presented for:

- TEAEs
- Serious Adverse Events (SAEs)

- TEAEs by severity (mild, moderate, severe, potentially life-threatening)
- TEAEs by relationship (Certain, probable, possible, not related)
- ADRs by severity
- TEAEs leading to drug interrupted
- TEAEs leading to drug withdrawn
- TEAEs leading to withdrawal

Listings will be made of all AEs, including non-TEAEs, SAEs, clinically significant AEs, ADRs including Suspected Unexpected Serious Adverse Reaction (SUSARs), and TEAEs leading to drug interruption or withdrawal from treatment.

7.2.2 Vital signs

Vital signs assessments are heart rate, oral temperature, systolic and diastolic blood pressure.

Vital signs will be summarised by visit including change from baseline. Boxplots will be presented by visit.

All data will be listed.

7.2.3 Laboratory Safety Data

Abnormal clinically significant laboratory should be reported as AEs, please refer to section 7.2.1 regarding the presentation of AEs.

Subject ID, visit number, collection date, reason for missed sample, and presence of abnormal and clinically significant laboratory values (yes/no) at the visit will be listed.

7.3 Analysis and Presentation of the Secondary Immunogenicity Endpoint

The secondary immunogenicity endpoint is seroconversion for anti-CTH522 IgG antibody at any time points after vaccinations.

All immunogenicity endpoints will be analysed using the FAS and the subjects will be evaluated according to the actual treatment received. The PP analysis set will be used as supportive.

7.3.1 Anti-CTH522 IgG Antibody

The anti-CTH522 IgG titre will be summarised by visit: Number of subjects with available titre, geometric mean, geometric coefficient of variation (CV), median, minimum, and maximum will be presented for actual values and ratio to baseline (fold difference).

Percentage of subjects achieving seroconversion for anti-CTH522 IgG at any time point after first vaccination will be presented for

- 4-fold increase compared to baseline
- 10-fold increase compared to baseline

The number and percentage of subjects achieving 4-fold increase respectively 10-fold increase seroconversion for anti-CTH522 IgG will be summarised by visit including cumulative numbers over

time. If a subject achieves seroconversion at a visit, the same subject will be counted as having seroconversion at all subsequent visits in the cumulative summary.

In addition to the descriptive presentation outlined above, the following analyses which are not described in the protocol will be presented:

The anti-CTH522 IgG titre will be compared by a non-parametric Mann-Whitney-Wilcoxon exact two-sample test for

- Cohort A-C vs Cohort D at Day 28
- Cohort A-C vs Cohort E at Day 28
- Cohort A vs Cohort B at Day 126
- Cohort A vs Cohort C at Day 126
- Cohort B vs Cohort C at Day 126
- Cohort A vs Cohort D at Day 126
- Cohort A vs Cohort E at Day 126

Using SAS© *Proc NPAR1WAY* with option *exact wilcoxon*.

If the value of anti-CTH522 IgG is below lower limit of quantification (LLOQ), then the value is set to LLOQ to be able to calculate the change.

The anti-CTH522 IgG titre will also be presented on a log scale in a spaghetti-plot by subject and day. The geometric mean will be presented in a plot by treatment group and day.

The cumulative proportion of subjects achieving 4-fold increase respectively 10-fold increase seroconversion for anti-CTH522 IgG will be presented in a plot by treatment group and day. In addition, a plot showing the median titre will be presented by treatment group and day.

All data will be listed.

7.4 Exploratory immunogenicity endpoints

The exploratory immunogenicity endpoints are

- Systemic and ocular antibodies
    - Cell-mediated immune response measured by Elispot and/or flow cytometry
    - Antibody responses measured by T- and B-cell Elispot
    - Serum neutralising antibodies against serovars D-G
- Isolation and characterisation of CTH522–antigen-specific memory B-cells in the systemic compartments (dependent on the elicited specific memory T- and B-cell numbers)

and will be handled in a separate report.

7.5 Interim Analysis

No interim analysis is planned.

7.6 Handling of Missing Values

No imputation of missing data is planned.

7.7 Multiplicity Adjustments

Several Fishers exact test will be calculated for the co-primary endpoints, and several nonparametric Wilcoxon tests will be calculated for the comparisons of anti-CTH522 IgG titre. They should all be interpreted with care when evaluating the results.

7.8 Sub-group and Centre Effects

No sub-group analyses are planned.

Adjustments for centre effects are not applicable in this single centre trial.

8 Deviations from Protocol

- The periods where the events for three of the co-primary endpoints (solicited local injection site reactions, solicited ocular reactions, and solicited systemic reactions) are considered were not specified in the protocol but in the subjects' diary. In this SAP the vaccination days are detailed as well as the 14-day period after vaccinations.

- For the co-primary endpoint *Solicited systemic reactions after IM and/or ID administration*, the PT *Pyrexia* should be used for temperatures above 38.3°C only, which is not as in the FDA guidelines presented in the protocol Appendix 1. Appendix 1 is stating temperatures above 38.0°C. For increased temperatures at or below 38.3°C, the PT *Body temperature increased* is used and thus not included as events. If the temperature is unknown, *Pyrexia* will be used, and the event included.
- The Wilcoxon tests of anti-CTH522 IgG titre are not described in the protocol.
- Presentations and analyses are adapted to fit the nature of the data.

9 Software

All statistical calculations described in this SAP will be done by [redacted] using SAS©, release 9.4 or later (SAS Institute, Cary, NC, USA). Tables, figures, and listings will be presented in landscape using size *Letter*.