PROTOCOL

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

SSI trial Code: CHLM-02

EudraCT No.: 2019-001090-88

ClinicalTrial.gov No.: NCT03926728

Trial phase: I

Version: Final 5.1

Date: 14 December 2020

Clinical trial manager:

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Vaccine Development Department
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Denmark

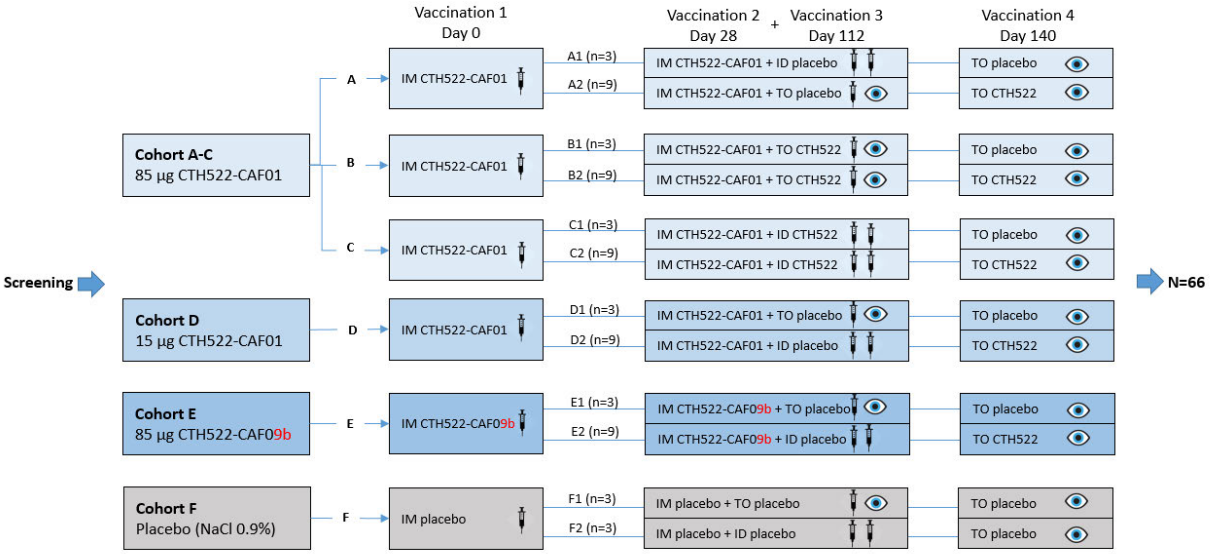
1 Synopsis

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
Trial code	CHLM-02
EudraCT	2019-001090-88
ClincialTrial.gov	NCT03926728
Trial phase	Phase I
Trial period	Q3 2019–Q4 2020
Principal investigator(s)	[redacted], MD, NIHR Imperial Clinical Research Facility, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London
Investigational site(s)	NIHR Imperial Clinical Research Facility Hammersmith Hospital, Imperial College Healthcare NHS Trust, Du Cane Road, London W12 0HS
Primary objective	• To evaluate the safety of CTH522-CAF01 administered IM in different doses • To evaluate the safety of non-adjuvanted CTH522 administered TO or ID simultaneously with CTH522-CAF01 IM • To evaluate the safety of CTH522-CAF09b administered IM
Secondary objective(s)	• To evaluate the serum 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 administered on Day 140
Exploratory objective(s)	• To evaluate the systemic and mucosal immunogenicity after vaccination with CTH522 with or without different adjuvants and administration routes
Primary endpoint	• Solicited local injection site reactions after ID and/or IM administration of the vaccines: erythema, pruritus, pain, tenderness, swelling, and warmth • Solicited local reactions after TO administration of the vaccine: watering eyes, swelling of eyelid, eye redness, and eye discomfort • Solicited systemic reactions after IM and/or ID administration of the vaccines: oral temperature > 38.3°C, chills, myalgia and rash • Any other adverse events (AEs)
Secondary endpoints	• Seroconversion for anti-CTH522 IgG at any time points after vaccination of CTH522

Exploratory endpoints	• 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)

Trial design	This is a phase I, double-blind, parallel, randomised, and placebo-controlled trial of the chlamydia vaccine, CTH522, in healthy adults. It is planned to randomly assign 66 subjects into 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 (see figure below). All subjects in the active groups (cohort A-E) will receive three IM injections of the adjuvanted CTH522 and some (cohort B and C) will received the non-adjuvanted CTH522 via the TO or ID route (given at the same time as the 2nd and 3rd IM vaccinations). All active groups will receive TO administration as a boost at Day 140 of either the non-adjuvanted CTH522 (12 µg in each eye) or placebo. The enrolled subjects will complete 12 trial visits (Vs): • V1 (Day -90 to -1): eligibility is assessed and informed consent is obtained; medical history, vital signs (including BMI) are taken; physical and eye examinations are performed: concomitant medications (CM) and AEs are recorded; blood samples for screening of hepatitis B and C, HIV, syphilis and safety biochemistry/haematology are drawn; urine is collected for pregnancy test and PCR for *C. Trachoma* and gonorrhoea infections • V2 (Day 0): eligibility is assessed; eye and symptom-direct physical examination are performed and vital signs are taken; AEs and CMs are reviewed and recorded; urine is collected for pregnancy test; randomisation; blood and ocular samples for safety and immunogenicity determination before vaccination; 1st IM vaccination; subjects observed for any immediate AE for 60 minutes after the vaccination; diary card is issued • V3 (Day 14): diary card reviewed; AEs and CM recorded; vital signs are taken and symptom-directed physical examination is performed; blood samples for safety and immunogenicity are drawn • V4 (Day 28): AEs and CM recorded; vital signs are taken; eye and symptom-directed physical examinations are performed; blood samples for safety and immunogenicity determination taken before vaccination; urine for pregnancy and ocular samples are taken; 2nd IM vaccinations together with ID or TO vaccination; subjects observed for any immediate AE for 60 minutes after the vaccination; diary card is issued • V5 (Day 42): diary card reviewed; AEs and CM are recorded; vital signs and ocular samples are taken; eye and symptom-directed physical examination are performed; blood samples for safety and immunogenicity are drawn • V6 (Day 56): AEs and CM are recorded; eye and symptom-directed physical examination are performed; blood samples for immunogenicity determination are drawn; ocular sample is taken • V7 (Day 112): AEs and CM are recorded; vital signs are taken; eye and symptom-directed physical examinations are performed; blood samples for safety and immunogenicity determination are drawn before vaccination; urine for pregnancy and ocular samples are taken; 3rd IM vaccinations together with ID or TO vaccination; subjects are observed for any immediate AE for 60 minutes after the vaccination; diary card is issued • V8 (Day 126): diary card is reviewed; AEs and CM are recorded; vital signs are taken, eye and symptom-directed physical examination are performed; blood samples for safety and immunogenicity determination are drawn; ocular sample is taken • V9 (Day 140): AEs and CM are recorded; vital signs are taken; eye and symptom-directed physical examinations are performed; blood samples for safety and immunogenicity determination are drawn before vaccination; urine for pregnancy and ocular sample are take; final vaccination with a TO CTH522 (or placebo) boost; subjects are observed for any immediate AE for 60 minutes after the vaccination; diary card is issued • V10 (Day 143): diary card is reviewed; AEs and CM are recorded; vital signs are taken; eye and symptom-directed physical examination are performed; blood samples for immunogenicity assessment are drawn and ocular sample is taken

	• V11 (Day 154): diary card is reviewed, AEs and CM are recorded; vital signs are taken; eye and symptom-directed physical examinations are performed; blood samples for safety and immunogenicity determination are drawn and ocular sample is taken • V12 (Day 238): end-of-trial visit; AEs and CM are reviewed and recorded; vital signs are taken; eye, physical examinations are performed; urine is collected for pregnancy and PCR for *C. Trachoma* and gonorrhoea infection test; blood samples for safety and immunogenicity determination are drawn and ocular sample is taken



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

Trial population and planned sample size	The trial plans to screen approximately 110 subjects with the aim to randomise and enrol 66 subjects. The enrolled subjects will be healthy male and female volunteers aged 18–45 years.
Inclusion and exclusion criteria	Inclusion criteria: • IC1: Healthy males and females between 18–45 years old on the day of the first vaccination • IC2: Has been properly informed about the trial and signed the consent form • IC3: Is willing and likely to comply with trial procedures • IC4: Is prepared to grant authorised persons access to his/her trial-related medical record • IC5: Is willing to use acceptable contraceptive measures during the trial (two weeks before and two weeks after the trial). Heterosexually active female capable of becoming pregnant must agree to use hormonal contraception, intrauterine device, intrauterine hormone-releasing system, or to complete abstinence from at least two weeks before the first vaccination until at least two weeks after the last. Complete abstinence (defined as refraining from heterosexual intercourse) must be in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), withdrawal and progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action are not acceptable methods of contraception Exclusion criteria: General • EX1: Is positive for *C. trachomatis* via urine PCR or has a known history of *C. trachomatis* • EX2: Is positive for gonorrhoea via urine PCR test, or HIV, hepatitis B/C, syphilis via blood tests

	• EX3: Has a significant active disease such as cardiac, liver, immunological, neurological, psychiatric or clinically significant abnormality of haematological or biochemical parameters • EX4: Has BMI ≥ 35 kg/m^2 • EX5: Is currently participating in another clinical trial with an investigational or non-investigational drug or device, or was treated with an investigational drug within 28 days before the first vaccination • EX6: Has received, or plans to receive, any immunisation within 14 days of the start of the trial or during the trial immunisations • EX7: Is currently receiving treatment with systemic immunosuppressive agents. Topical steroids are allowed unless applied to the IM or ID injection site • EX8: Has a condition which in the opinion of the investigator is not suitable for participation in the trial • EX9: Is known or confirmed to have allergy to any of the vaccine constituents • EX10: Is unable to refrain from use of contact lenses. Contact lenses should be avoided two days before TO administration and for seven days later (longer if any ongoing local eye AE) • EX11: Has any evident ocular disease upon eye exam (prior to first vaccination) or any medical history of ocular disease that, in the opinion of the investigator, may impact the subject's participation in the trial Female-specific • EX12: Is pregnant (positive pregnancy test) or breastfeeding or not willing to use contraception during the trial • EX13: Has confirmed history of pelvic inflammatory disease or significant gynaecological diseases
Investigational medicinal products	The dosages for the investigational medicinal products (IMPs) are presented in the table below. Saline 0.9% NaCl solution for placebo are to be administered IM, TO and ID. The non-adjuvanted CTH522 to be given TO is ready to be used. The CTH522-CAF01 and CTH522-CAF09b (both to be given IM) and CTH522 to be given ID will be reconstituted at the site. **IMP (routes)** / **CTH522** / **Per dose volume** CTH522-CAF01 (IM) / 85 µg / 0.5 ml CTH522-CAF01 (IM) / 15 µg / 0.5 ml CTH522-CAF09b[1] (IM) / 85 µg / 0.5 ml CTH522 (TO) / 24 µg[1] / 0.04 ml CTH522 (ID) / 24 µg / 0.1 ml [1]subjects receive 12 µg in each eye.
Dosages and route of administration	The preferred location for IM and ID administrations is into (IM) or into the skin overlying (ID) the non-dominant deltoid muscle. Each IM administration will consist of 85 µg CTH522-CAF01, 15 µg CTH522-CAF01 or 85 µg CTH522-CAF09b in a volume of 0.5 ml per injection. Each ID administration will consist of 24 µg CTH522 in a volume of 0.1 ml per injection. The IM and ID injections must be administered within five minutes of each other and approximately 2 cm apart from each other. As long as the two injections are administered within five minutes, it does not matter which one (IM or ID) is given first. The IM injection will be performed with a 1–2 ml polypropylene syringe via a 23–25-gauge needle. The ID injection will be with a 1 ml syringe via a microneedle device. The TO administration in both eyes will consist of 24 µg CTH522 (non-adjuvanted) in a volume of 0.04 ml; thus, each eye is administered 12 µg of CTH522 TO in a 0.02 ml drop. The eye drops should be given five minutes apart, with the right side first to follow by the left side. The TO administrations will be performed using a Gilson MICROMAN positive displacement pipette.

Statistical methods	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. The basis for safety analysis is the safety analysis set (SAF), which consists of all subjects randomised and exposed to the IMPs. Full analysis set (FAS) will include subjects with observed data for all randomised subjects. A per-protocol analysis set (PP) will include subjects who completed the trial without deviations judged to influence the primary endpoints. The primary analysis will be carried out for SAF and FAS and PP. Categorical data will be summarised descriptively by treatment, using the number and percentages of subjects. Continuous data will be presented using the number of subjects, mean, standard deviation, median, minimum and maximum. Both the absolute values and the change from baseline will be presented. Primary efficacy data will be presented descriptively over time using box-plots over time. If the nature of data allow, a formal statistical test will be performed using time to event analysis.

AE=adverse event; CM=concomitant medication; FAS=full analysis set; ID=intradermal; IM=intramuscular; IMP=investigational medicinal product; PP=per-protocol analysis set; SAF=safety analysis set; TO=topical ocular; V=visit

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3 List of Abbreviations and Definitions

AE	Adverse event
$Al(OH)_3$	Aluminium hydroxide used in CHLM-01 trial as an adjuvant combined to CTH522
ALT	Alanine aminotransferase
BMI	Body mass index
C. trachomatis	Bacterium *Chlamydia trachomatis* can cause trachoma, contagious inflammatory disease of the eye
CA	Competent authority
CAF01	Adjuvant promotes both strong neutralising antibody titres and Th1 responses. Combined with CTH522, CAF01 is used in the CHLM-02 trial
CAF09b	Adjuvant that, when formulated with other vaccines, resulted in a high frequency of antigen-specific CD8+ T-cells (29). Combined with CTH522, CAF09b is used in the CHLM-02 trial
CI	Confidence Intervals
CIOMS form	Serious adverse event is reported via the Council for International Organisations of Medical Sciences form and sent to the authority
CM	Concomitant medication
CRA	Clinical Research Associate
CRO	Contract research organisation
CTH522	Non-adjuvanted chlamydia antigen
CTH522-CAF01	Chlamydia antigen combined with adjuvant CAF01, an on-site reconstituted IMP used in CHLM-02
CTH522-CAF09b	Chlamydia antigen combined with adjuvant CAF09b, an on-site reconstituted IMP used in CHLM-02
CTR	Clinical trial report
DDA	Dimethyldioctadecylammonium bromide
EC	Ethics committee
eCRF	Electronic Case Record Form
EDC	Electronic data capture (system used for electronic recording in the eCRF)
ELISA	Enzyme-linked immunosorbent assay
Elispot	Enzyme-linked immunospot assay
EX	Exclusion criteria
FAS	Full analysis set
FDA	Food and Drug Administration
FSFV	First subject's first visit
GCP	Good clinical practice according to ICH GCP E6
GMP	Good manufacturing practice

HIV	Human immunodeficiency virus
HRA	Health Research Authority
IB	Investigator's brochure
IC	Inclusion criteria
ICF	Informed consent form
ICH	International Council for Harmonisation
ICHT	Imperial College Healthcare NHS Trust (investigator site)
ICL	Imperial College London
ICS	Intracellular cytokine staining
ID	Intradermal
IF	Investigator's file
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IM	Intramuscular
IMP	Investigational medicinal product. The non-adjuvanted CTH522 to be given TO is ready to be used IMP. The CTH522-CAF01 and CTH522-CAF09b (both to be given IM) and CTH522 (to be given ID) are reconstituted at the site
LSLV	Last subject's last visit
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency
MMG	Monomycoloyl glycerol analogue
MOMP	Major outer membrane molecule
NIHR	National Institute for Health Research
PBMC	Peripheral blood mononuclear cell(s)
PCR	Polymerase chain reaction
PI	Principal investigator
Poly I:C	Polyinosinic:polycytidylic acid
PP	Per-protocol set
PT	MedDRA preferred term
RBC	Red blood cells
SAE	Serious adverse event
SAF	Safety Analysis Set
SAP	Statistical analysis plan
SSI	Statens Serum Institut
SUSAR	Suspected unexpected serious adverse reaction
TDB	Trehalose dibehenate
TMF	Trial master file
TO	Topical ocular

V1, V2, V3, …	Trial Visit 1, Visit 2, Visit 3, …
VD4	Variable domains
WBC	White blood cell
WHO	World Health Organisation
WHO-DDE	World Health Organisation Drug Dictionary Enhance

4 Signature page

Principal investigator, Imperial College Healthcare NHS Trust: [redacted] [redacted] 16-Dec-20
date & signature

Non-clinical investigator, Imperial College London: [redacted] [redacted] 14.12.2020
date & signature

Trial statistician [redacted] [redacted]
date & signature

Sponsor's representative, SSI: [redacted] [redacted]
date & signature

Trial manager, SSI: [redacted] [redacted] 14-DEC-2020
date & signature

5 Relevant addresses

Relevant trial staff and personnel from clinical research organisations (CROs) are listed in Table 1. Contact information on the serious adverse event (SAE) reporting is listed in Table 2.

Table 1: Relevant trial staff and personnel

Principal Investigator (PI): [redacted]	NIHR Imperial Clinical Research Facility, Hammersmith Hospital, Imperial College Healthcare NHS Trust, Du Cane Road, London W12 0HS, United Kingdom	Phone: [redacted] Email: [redacted]
Non-clinical Investigator: [redacted]	Wright-Fleming Wing, St. Mary's Hospital Campus, Imperial College London, Norfolk Place, London W2 1PG, United Kingdom	Phone [redacted] Email: [redacted]
Clinical trial manager: [redacted]	Statens Serum Institut Vaccine Development Department 5 Artillerivej DK-2300 Copenhagen S	Phone: [redacted] Email: [redacted]
Pharmacovigilance manager: [redacted]	Statens Serum Institut Vaccine Development Department 5 Artillerivej DK-2300 Copenhagen S	Phone: [redacted] Email: [redacted]
Sponsors medical expert: [redacted]	Statens Serum Institut Infectious Disease Immunology 5 Artillerivej DK-2300 Copenhagen S	Phone: [redacted] Email: [redacted]
Sponsor's representative: [redacted]	Statens Serum Institut Vaccine Development Department 5 Artillerivej DK-2300 Copenhagen S	Phone: [redacted] Mobile: [redacted] Email: [redacted]
Trial statistician: [redacted]	[redacted] DK-	Phone: [redacted] Email: [redacted]
Pharmacovigilance specialist: [redacted]	[redacted] DK-	Phone: [redacted] Email: [redacted]

Table 2: Serious adverse event and medical emergency reporting contact information

SAE reports from investigational site to SSI and CRO:	SAE report via trial database. An email notification is sent to [redacted] and SSI [redacted]
Emergency phone numbers for subjects:	[redacted] (Monday–Friday 0900–1700) [redacted] (outside normal working hours)

6 Introduction

Trachoma is a contagious inflammatory disease of the eye following infection by a bacterium *Chlamydia trachomatis* (*C. trachomatis*). Although the disease is preventable, the infection caused blindness in 1.9 million people worldwide according to WHO fact sheet from 2019 [1]. Classical control measures such as diagnosis, screening programs, and treatment have been intensified in recent years, but the number of *C. trachomatis* infections is still increasing. As these infections are not effectively controlled, an effective vaccine is required [2, 3].

The TracVac Consortium consisting of Imperial College London (ICL) in the UK, Statens Serum Institut (SSI) in Denmark, London School of Hygiene & Tropical Medicine in the UK and The French Alternative Energies and Atomic Energy Commission (CEA) in France plan to conduct this phase I trial with a vaccine (CTH522) against trachoma following successful pre-clinical studies.

Statens Serum Institut has developed the novel recombinant vaccine antigen (CTH522, 53.9 kDa). It is a recombinant version of the major outer membrane molecule (MOMP) from *C. trachomatis*, which is designed to induce broadly neutralising antibodies. CTH522 is highly immunogenic and contains conserved T-cell epitopes, frequently recognized by *C. trachomatis* infected patients, combined with B-cell epitopes spanning the protective VD4 region in MOMP from the four most frequent human *C. trachomatis* serovars [4, 5].

CTH522 protects against genital *C. trachomatis* infection in the mouse model and is highly immunogenic in mice, rabbits, guinea pigs and minipigs [4]. There was no evidence of a broader toxic effect of the adjuvanted CTH522-CAF01 and CTH522-$Al(OH)_3$ when administered intramuscularly (IM) and boosted intranasally with non-adjuvanted CTH522 to Gottingen minipigs in a good laboratory practice-compliant repeat dose study [6].

CTH522 was also evaluated in the EpiOcular *In vitro* test provided by MatTek. The MatTek EpiOcular™ model is a commercially available 3D model of the human corneal epithelium, derived from normal human epidermal keratinocytes. The cells have been cultured *in vitro* to form a multi-layered structure which closely resembles the corneal epithelium of the human eye. The EpiOcular™ *In Vitro* test categorised CTH522 600 Test Item as “no category”, which were considered as non-irritant if the tissue viability after exposure is >60%. Hence CTH522 is concluded to be non-irritant to the eye [7].

Following the EpiOcular *In vitro* test, a study investigating safety and immunogenicity of CTH522 with or without the adjuvant CAF01 or CAF09b administered IM or in combination of topical ocular (TO) and intradermal (ID) was conducted in 36 cynomolgus macaques. The study found no major clinical observation after vaccination and concluded that the adjuvants were safe for the tested animals. Therefore, this study supports the suitability of CTH522-CAF01 and CTH522-CAF09b as safe, immunogenic, and potentially effective vaccines to induce immunity in *C. trachomatis* uninfected individuals [8].

A first-in-man clinical trial with 35 volunteers using CTH522 took place at Imperial College Healthcare NHS Trust, London during 2016–2017 (CHLM-01, EudraCT 2015-004330-10). The volunteers received three IM (CTH522 adjuvanted with CAF01 or $Al(OH)_3$) administrations at Day 0, 28, and 112, followed by two intranasal (non-adjuvanted) at Day 126 and 140. Based on the safety results of this phase I trial, the tested antigen CTH522 is considered safe when adjuvanted with $Al(OH)_3$ or CAF01. All actively vaccinated subjects achieved seroconversion (both 2- and 4-fold) whereas none of the subjects in the placebo group did; hence the CTH522 showed to be immunogenic when combined with either $Al(OH)_3$ or CAF01 adjuvants. Furthermore, after the third vaccination and onwards, CAF01 enhanced the CTH522-specific IgG response statistically significantly more than $Al(OH)_3$ in the trial population [9].

In the present CHLM-02 trial, the CTH522 antigen will be administered non-adjuvanted or adjuvanted with CAF01 or CAF09b. The CAF01 adjuvant promotes both strong neutralising antibody titres and Th1 responses in mice, pigs and non-human primates [4, 10, 11]. Furthermore, CAF01 has proven safe and immunogenic in other clinical trials such as with the tuberculosis vaccine Ag85B-ESAT-6:H1[12], first-in-human chlamydia vaccine CTH522 [9] and malaria vaccine (GLURP-MSP3) [13].

The CAF09b is a novel adjuvant that, when formulated with other vaccines, resulted in a high frequency of antigen-specific CD8+ T-cells in animal study [14]. CAF09b is a cationic liposomal adjuvant consisting of the cationic lipid dimethyldioctadecylammonium bromide (DDA) and a monomycoloyl glycerol analogue (MMG) with the TLR3 agonist polyinosinic:polycytidylic acid (poly I:C)) electrostatically adsorbed to the surface of the liposomes. Based on animal toxicity studies, CAF09b is expected to be safe and tolerable in humans as well. Currently, two investigator-initiated trials (ClinicalTrial.gov NCT03412786 and NCT03715985) with CAF09b-adjuvanted vaccines are being conducted in cancer patients in Denmark. As of 02 May 2019, 18 subjects have been enrolled between the two trials where 30 IM and 25 intraperitoneal vaccinations with a CAF09b-containing vaccine have been administered; no SAEs have been reported.

On the basis of the results of these clinical and non-clinical studies described above, it is considered safe to start a clinical phase I trial with CTH522-CAF01 and CTH522-CAF09b as well as non-adjuvanted CTH522 in healthy adult volunteers. The vaccines in the CHLM-02 trial will be given IM, ID and TO. The main purpose of including TO and ID administrations in addition to the IM is to test if these vaccination routes demonstrate a higher ability to facilitate an ocular immune response than the IM vaccinations alone. A boost of CTH522 given TO at Day 140 seeks to investigate if a TO only boost will increase the ocular immune response.

7 Trial objectives

7.1 Primary objectives

- To evaluate the safety of CTH522-CAF01 administered IM in different doses
- To evaluate the safety of non-adjuvanted CTH522 administered TO or ID simultaneously with CTH522-CAF01 IM
- To evaluate the safety of CTH522-CAF09b administered IM

7.2 Secondary objectives

- To evaluate the serum 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 if non-adjuvanted CTH522 on Day 140

7.3 Exploratory objectives

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

7.4 Primary endpoint

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

7.5 Secondary endpoint

- Seroconversion for anti-CTH522 IgG at any time points after vaccinations

7.6 Exploratory endpoints

- 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)

8 Investigational plan

8.1 Overall trial design

The present 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 (ICHT), London, in the UK.

It is planned to randomly assign 66 subjects into 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 (Vs) (Table 3).

All subjects in the active groups (cohort A-E) will receive three IM injections of the adjuvanted CTH522 and some (cohort B and C) will received the non-adjuvanted CTH522 via the TO or ID route (given at the same time as the 2nd and 3rd IM vaccinations). All active groups will receive TO administration as a boost at Day 140 of either the non-adjuvanted CTH522 (12 µg in each eye) or placebo.

- Cohort A will receive three IM vaccination of 85µg CTH522-CAF01. This cohort is divided into two groups: A1 will receive ID placebo at Day 28 + Day 112, and TO placebo at Day 140, while A2 will receive TO placebo at Day 28 + Day 112, and non-adjuvanted TO CTH522 boost at Day 140.

- Cohort B will receive three IM vaccinations of 85 µg CTH522-CAF01. This cohort is divided into two groups: B1 will receive TO vaccination of the non-adjuvanted CTH522 at Day 28 and 112 and TO placebo at Day 140, while B2 will receive the same for Day 28 and 112, but non-adjuvanted TO CTH522 boost at Day 140. The two additional TO doses of CTH522 (12 µg in each eye) are administered in each eye. The rationale for this schedule is to investigate the impact of simultaneous TO administration of the antigen on the immunogenicity results.

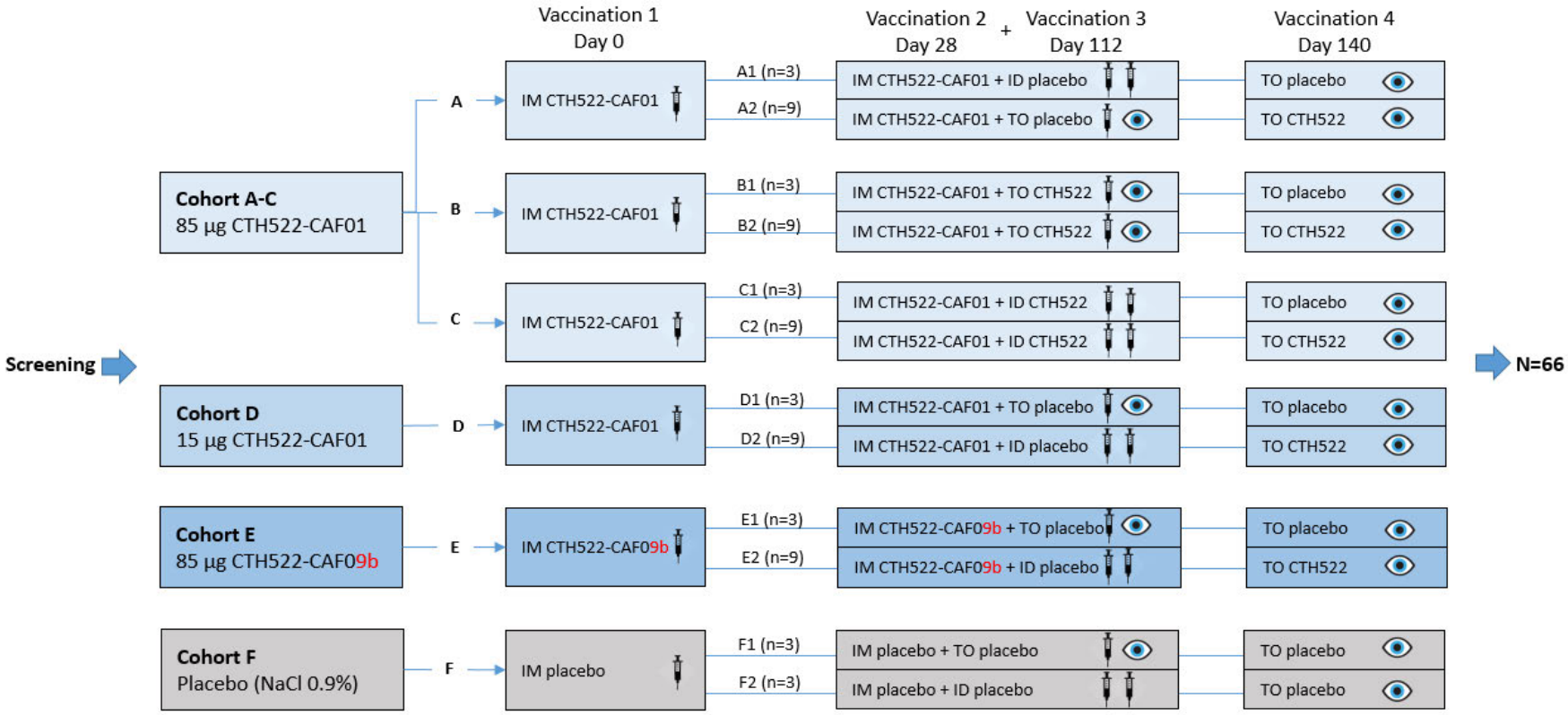
- Cohort C will receive three IM vaccinations of 85 µg CTH522-CAF01. This cohort is divided into two groups: C1 will receive ID vaccination of the non-adjuvanted 24 µg CTH522 at Day 28 and 112 and TO placebo at Day 140, while C2 will receive the same for Day 28 and 112, but TO 12 µg CTH522 boost in each eye at Day 140. The rationale for this schedule is to investigate the impact of simultaneous ID administration of the antigen on the immunogenicity results.

- Cohort D will receive three IM vaccinations of 15 µg CTH522-CAF01. The rationale for the A and D cohorts is to investigate the impact of the two IM CTH522 doses on the immunogenicity results.

- Cohort E will receive three IM vaccinations of 85 µg CTH522-CAF09b. The rationale for the A and E cohorts is to investigate the impact of the adjuvant on the immunogenicity results.

- Cohort F will receive only placebo in form of 0.9% NaCl (IM, ID and TO).

Figure 1: Trial diagram



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 3: Trial 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

8.2 Trial procedures

At V1 (Day -90 to -1), the volunteers' eligibility is assessed according to the pre-specified inclusion/exclusion criteria and the informed consent is obtained. The medical history, vital signs (including BMI) are taken, physical and eye examinations are performed. Refer to Table 3 for eye examination method required at V1. Concomitant medications (CM) and AEs are recorded. Blood samples for screening of hepatitis B and C, HIV, syphilis and safety biochemistry/haematology are drawn. Urine is collected for pregnancy test and PCR for *C. Trachoma* and gonorrhoea infections.

At V2 (Day 0), the inclusion/exclusion criteria are assessed again. Eye and symptom-direct physical examination are performed and vital signs are taken. Refer to Table 3 for eye examination method required at V2. Any AEs and CMs are reviewed and recorded. Urine is collected for pregnancy test. The volunteers are randomised according to the randomisation scheme via the eCRF. Before the 1st IM vaccination, blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn and ocular samples are taken. The volunteers (hereafter subjects) receive the 1st IM vaccination. The subjects are observed for any immediate AE for 60 minutes after the vaccination. The diary card is issued with instructions before discharge from the site.

At V3 (Day 14), the diary card is collected and reviewed. Adverse events and CM are reviewed and recorded. Vital signs are taken and symptom-directed physical examination is performed as necessary. Blood samples for safety and immunogenicity (please refer to type of tests in Table 4) are drawn.

At V4 (Day 28), the AEs and CM are reviewed and recorded. Vital signs are taken. Eye and symptom-directed physical examinations are performed. Refer to Table 3 for eye examination method required at V4. Before the vaccinations, blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn, and urine for pregnancy and ocular samples are taken. The subjects receive the 2nd IM vaccinations together with ID or TO vaccination as specified in the Figure 1. In the event that the first TO vaccinations are planned for several subjects on the same day, there should be a minimum of two hours between the TO dosing for each subject. The two hour lagging between the TO doses should be performed for the first five subjects receiving TO doses and can be discontinued after the first these five subjects if there are no safety concerns. The subjects are observed for any immediate AE for 60 minutes after the vaccination. The diary card is issued with instructions before discharge from the site.

At V5 (Day 42), the diary card is collected and reviewed, AEs and CM are reviewed and recorded. Vital signs and ocular samples are taken. Eye and symptom-directed physical examination are performed. Refer to Table 3 for eye examination method required at V5. Blood samples for safety and immunogenicity (please refer to type of tests in Table 4) are drawn.

At V6 (Day 56), the AEs and CM are recorded. Eye and symptom-directed physical examination are performed. Refer to Table 3 for eye examination method required at V6. Blood samples for immunogenicity determination are drawn (please refer to type of tests in Table 4). Ocular sample is taken.

At V7 (Day 112), the AEs and CM are recorded. Vital signs are taken. Eye and symptom-directed physical examinations are performed. Refer to Table 3 for eye examination method required at V7. Before the vaccinations, blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn, and urine for pregnancy and ocular samples are taken. The subjects receive the 3rd IM vaccinations together with ID or TO vaccination as specified in the Figure 1. The subjects are observed for any immediate AE for 60 minutes after the vaccination. The diary card is issued with instructions before discharge from the site.

At V8 (Day 126), the diary card is collected and reviewed. The AEs and CM are recorded. Vital signs are taken, eye and symptom-directed physical examination are performed. Refer to Table 3 for eye examination method required at V8. Blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn. Ocular sample is taken.

At V9 (Day 140), the AEs and CM are recorded. Vital signs are taken. Eye and symptom-directed physical examinations are performed. Refer to Table 3 for eye examination method required at V9. Before the vaccinations, blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn, urine for pregnancy and ocular sample are taken. The subjects receive the final vaccination with a TO CTH522 (or placebo) boost as specified in the Figure 1. The subjects are observed for any immediate AE for 60 minutes after the vaccination. The diary card is issued with instructions before discharge from the site.

At V10 (Day 143), the diary card is reviewed. The AEs and CM are recorded. Vital signs are taken. Eye and symptom-directed physical examination are performed. Refer to Table 3 for eye examination method required at V10. Ocular sample is taken.

At V11 (Day 154), the diary card is collected and reviewed, AEs and CM are recorded. Vital signs are taken. Eye and symptom-directed physical examinations are performed. Refer to Table 3 for eye examination method required at V11. Blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn and ocular sample is taken.

V12 (Day 238) is the end-of-trial visit. The AEs and CM are reviewed and recorded. Vital signs are taken. Eye and physical examinations (including symptom-directed) are performed. Refer to Table 3 for eye examination method required at V12. Urine is collected for pregnancy and PCR for *C. Trachoma* and gonorrhoea infection test. Blood samples for safety and immunogenicity determination (please refer to type of tests in Table 4) are drawn and ocular sample is taken.

8.3 Trial population and planned number of subjects

The trial plans to screen approximately 110 subjects with the aim to randomise 66 subjects. The enrolled subjects will be healthy males and females aged 18–45 years.

8.3.1 Inclusion criteria

- IC1: Healthy males and females between 18–45 years old on the day of the first vaccination

- IC2: Has been properly informed about the trial and signed the consent form
- IC3: Is willing and likely to comply with trial procedures
- IC4: Is prepared to grant authorised persons access to his/her trial-related medical record
- IC5: Is willing to use acceptable contraceptive measures during the trial (two weeks before and two weeks after the trial). Heterosexually active female capable of becoming pregnant must agree to use hormonal contraception, intrauterine device, intrauterine hormone-releasing system, or to complete abstinence from at least two weeks before the first vaccination until at least two weeks after the last. Complete abstinence (defined as refraining from heterosexual intercourse) must be in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), withdrawal and progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action are not acceptable methods of contraception

8.3.2 Exclusion criteria

General

- EX1: Is positive for *C. trachomatis* via urine PCR or has a known history of *C. trachomatis*
- EX2: Is positive for gonorrhoea via urine PCR test, or HIV, hepatitis B/C, syphilis via blood tests
- EX3: Has a significant active disease such as cardiac, liver, immunological, neurological, psychiatric or clinically significant abnormality of haematological or biochemical parameters
- EX4: Has BMI ≥ 35 kg/m^2
- EX5: Is currently participating in another clinical trial with an investigational or non-investigational drug or device, or was treated with an investigational drug within 28 days before the first vaccination
- EX6: Has received, or plans to receive, any immunisation within 14 days of the start of the trial or during the trial immunisations
- EX7: Is currently receiving treatment with systemic immunosuppressive agents. Topical steroids are allowed unless applied to the IM or ID injection site
- EX8: Has a condition which in the opinion of the investigator is not suitable for participation in the trial
- EX9: Is known or confirmed to have allergy to any of the vaccine constituents

- EX10: Is unable to refrain from use of contact lenses. Contact lenses should be avoided two days before TO administration and for seven days later (longer if any ongoing local eye AE)

- EX11: Has any evident ocular disease upon eye examination (prior to first vaccination) or any medical history of ocular disease that, in the opinion of the investigator, may impact the subject's participation in the trial

Female-specific

- EX12: Is pregnant (positive pregnancy test) or breastfeeding or not willing to use contraception during the trial

- EX13: Has confirmed history of pelvic inflammatory disease or significant gynaecological diseases

8.4 Randomisation procedure

The randomisation will be performed via the eCRF system at the site. Randomisation data is kept strictly confidential, accessible only to authorised persons, until the time of unblinding. Only when the trial has been completed or for expedited purpose, and the database has been locked, the codes will be broken and made available for data analysis.

8.5 Blinding and unblinding

Blinding will be obtained by shielding the subjects from seeing the preparation of the trial drug and by having unblinded trial personnel not involved in any trial assessments (immunogenicity or safety) responsible for preparing and administering the trial drug. This unblinded staff will prepare and administer trial drug, and be the only ones doing IMP accountability.

During vaccination, the blinded staff will be shielded from seeing trial drug or any procedures related hereto. This blinded staff will observe the subject and monitor any AEs during or after vaccination.

All used material will be removed by the unblinded staff without revealing the identity of the fluid. The IMP accountability will be monitored by an unblinded clinical research associate (CRA).

The PI and delegates at the site will have access via the IBM Clinical Development trial database to unblind individual subjects and obtain information on trial drug administered in case of a medical emergency. The investigator should only unblind the treatment allocation to a subject during the clinical trial if it is relevant to the safety of the subject. The identity of the IMP would be revealed by the PI or designee for that subject only.

It may be necessary for the CRO to unblind a subject's treatment for expedited reporting to the Competent Authorities (CA) and/or Research Ethic Committee (EC). In these situations, the CRO will keep the unblinding from the PI, site staff, subject, and SSI personnel involved in monitoring, data analysis and interpretation. In cases of suspected unexpected adverse reactions (SUSAR), the unblinding will be known to the safety manager at SSI.

Unblinding of a subject is performed in the IBM Clinical Development trial database and is only possible for persons who have obtained rights to perform unblinding. The date of unblinding, reason and the person performing the unblinding are saved via an audit trail.

A screen-dump of the unblinding page including the information of the unblinded subject, the reason for unblinding, date and person performing the unblinding, is made and printed. This print is filed in an envelope with the subject's allocation number and sealed with a label and kept in a secure at the site.

The IBM Clinical Development system has an up-time of more than 99%, so system-down is a small risk. In case of system-down, a designated person at [redacted], who is not otherwise involved in any trial-related work, can be contacted to obtain information of the trial drug allocated to any specific subject.

Subjects with unblinded treatment allocation will not be withdrawn and will not be replaced.

8.6 Immunogenicity assessments

The immunogenicity objective, *anti-CTH522 IgG,* will be determined using ELISA at SSI.

The immunogenicity will further be analysed using a panel of exploratory assays performed at SSI and ICL as outlined in Table 4. The serum IgA ELISA + anti-CTH522 IgG subtypes + neutralising antibody test for the mucosal immune response, whereas the ocular anti-CTH522 IgG + IgA ELISA test the immune response in the eye. The T- and B-cell Elispot and ICS flow using frozen PBMCs assess the cell-mediated immunity. The transcriptional analysis consists of taking PBMCs and re-stimulating *in vitro* with CTH522. The aim is to identify gene transcription profiles associated with Th17 T-cells, Th1, and Th2 in the different active vaccine groups.

Table 4: Safety and immunogenicity assessments

Laboratory tests	Analysed at:	Medium, amount and unit	Visit	1	2	3	4	5	6	7	8	9	10	11	12
			Day	-	0	14	28	42	56	112	126	140	143	154	238
Safety															
Safety lab	ICHT	Blood 1 x 13 (8) ml		13	8	8	8	8		8	8	8		8	8
Immunogenicity															
Serum IgG ELISA[1]	SSI	Blood 1 x 10 ml			10	10	10	10	10	10	10	10		10	10
Serum IgA ELISA+ anti-CTH522 IgG subtypes + neutralising antibodies[2]	SSI	Blood 1 x 10 ml			10	10	10	10	10	10	10	10		10	10
T- and B-cell Elispot frozen PBMCs[2]	T-cell: SSI B-cell: ICL	Blood in heparin tube 5 x 6 ml			30	30	30	30	30	30	30	30		30	30
ICS flow frozen PBMCs[2]	ICL	Blood in heparin tube 5 x 6 ml			30	30	30	30	30		30	30		30	30
Transcriptional analysis frozen PBMCs[2]	SSI	Blood in heparin tube 2 x 6 ml			12						12			12	
Total blood collection															
For immunogenicity					92	80	80	80	80	50	92	80		92	80
For safety				13	8	8	8	8		8	8	8		8	8

ICHT=Imperial College Healthcare NHS Trust; ICL=Imperial College London; ICS=intracellular cytokine staining; PBMC=peripheral blood mononuclear cell; SSI=Statens Serum Institut

[1]for secondary endpoints

[2]for exploratory endpoints

8.7 Safety assessments

The trial staff must record all AEs, non-serious and serious, expected and unexpected in the relevant form of the eCRF. This starts from the time after the subject has signed the informed consent form (ICF) at V1 until the end-of-trial at V12.

The source of AEs covers, for example, the subject's response to questions about his/her health (a standard non-leading question), information from diary card, symptoms spontaneously reported by a subject, results from pre-defined laboratory testing and examinations where the findings are assessed by the investigator to be clinically significant changes or abnormalities, and other information relating to the subject's health becoming known to the investigator (for example hospitalisation).

The subjects will remain at the site for 60 minutes after each vaccination so that any immediate AEs observed may be recorded. It is also documented if no immediate AEs occurred. Trial staff will ensure that the subjects have a diary card, ruler to measure and thermometer to enable them to record AEs while away from the clinical site.

From the day of vaccination until seven days later, solicited AEs and unsolicited AEs will be recorded through the use of diary cards. Between Day 7–14, the subject will be asked to fill in details in the diary *only* if they experience an AE or if any medications were taken.

8.8 Predetermined reasons for discontinuation

The trial may be terminated at any time if participation in the trial is no longer considered safe by the PI, the sponsor or the regulatory authorities.

A subject is free to discontinue from the trial at any time without giving a reason. The investigator may at any time withdraw a subject if his/her participation is no longer considered safe or relevant. The date and reason (if given) for discontinuation must be recorded in the eCRF. In the case of pregnancy and/or anaphylactic reactions, the subject must be withdrawn. In case of an identified pregnancy, the pregnancy form(s) is to be used. If a subject for any reason leaves or is withdrawn from the trial or has a significant protocol deviation, he/she will not be replaced if the subject has received at least one trial vaccination.

Because the healthy subjects gain no benefit from participation in the trial, dosing in the trial must be stopped if:

- there is one or more subjects with SAEs considered to be related to the IMP
- there are two or more subjects with a severe AE considered to be at least possibly related to the IMP (based on the judgement of the investigator)
- there are two or more subjects with clinically significant AEs considered to be at least possibly related to the IMP (based on the judgement of the investigator) and which are

deemed to have impacted to a significant degree on the safety or physical or mental integrity of the affected subject(s), or which have resulted in permanent discontinuation of dosing in the affected subject(s)

8.9 Contraindications

8.9.1 Temporary contraindications

The below conditions (events) are considered temporary contraindications to the trial products and are assessed before each vaccination is administered.

- Oral temperature > 38.3°C measured immediately before each vaccination
- Acute illness as judged by the investigator
- Antipyretics used on the day of vaccination
- Systemic treatment with immunosuppressive agents
- Ongoing conjunctivitis or similar on the day of TO administration

If the subject is experiencing one of the above conditions, the vaccination must be postponed until he/she has recovered. On the rescheduled visit, the subject should be checked for the above temporary contraindications. If the subject is found to be eligible, the vaccine can be administered while maintaining the planned intervals.

8.9.2 Permanent contraindications

If the subject experienced the following events or received the following medication, he/she must be withdrawn from the trial:

- acute anaphylactic reaction after administration of any of the trial vaccines
- ocular disease

8.10 Collection and handling of samples

Detailed information on the collection and handling of blood, ocular strips and frozen PBMC will be described in Laboratory Manuals. During the trial, approximately 85 ml of blood will be collected for laboratory screening and safety tests, and approximately 800 ml of blood will be collected for immunogenicity assessments (Table 4)

8.11 Laboratory assays

Testing for *C. trachomatis* infection, gonorrhoea, syphilis, HIV, hepatitis B and C will be done at the laboratory facility at Charing Cross Hospital, which is part of ICHT.

Laboratory safety tests will be analysed at the lab facility at Hammersmith Hospital, which is also part of ICHT. Peripheral blood will be collected and analysed for the following biochemistry and haematology parameters at the timepoints specified in Table 3:

- Renal urea and electrolytes profile (sodium, potassium, chloride, urea, creatinine)
- Liver function tests (ALT, alkaline phosphatase, total bilirubin, total protein, albumin)
- Full blood count: WBC, lymphocytes, monocytes, eosinophils, neutrophils, basophils, RBC, haemoglobin, haematocrit, and platelets)

FDA guidelines on toxicity grading scale for subjects enrolled in vaccine trials will be used by the investigator for grading the intensity of AEs including lab-related AEs (Appendix 1).

A pregnancy test will be performed on-site by analysis of a urine sample for human chorionic gonadotrophin collected from female subjects at screening, the day of the vaccination and the final visit. Testing will be done at the NIHR Imperial Clinical Research Facility.

Serum IgG samples will be analysed at SSI. Other exploratory immunogenicity analysis will be analysed as specified in Table 4. Samples for PBMC isolation will be shipped on the day of collection from the investigator site to ICL, for processing and storage. The serum IgG samples will be processed and stored at the investigator site until the last sample has been collected. All samples intended to be analysed at SSI will be shipped after the last subject's last visit (LSLV) but no later than two weeks after date of LSLV. The samples must be shipped on dry ice with storage conditions monitored during the transport to SSI by use of a temperature logger. Documentation of the dispatch of samples from the investigational site and the receipt of the samples at SSI should be in accordance with the relevant SSI procedures for dispatch and receipt of samples. It is the responsibility of the site to pack the samples according to the above and arrange the transport from the site to SSI.

8.12 Planned schedule of trial

The schedule below is subject to change. The date of the first subject's first visit (FSFV) is defined as the date the first subject has had his/her first trial visit. The end of the trial is defined as LSLV, and the EC and MHRA will be notified within 90 days of the date of LSLV.

- Planned submission of clinical trial application to MHRA and EC: Q2 2019
- Planned FSFV: Q3 2019
- Planned LSLV: Q4 2020
- Database lock: Q1 2021
- Planned final integrated clinical trial report (CTR): Q1 2021

9 Investigational medicinal products

9.1 Composition of trial products

The drug products CTH522 and adjuvant CAF01 were manufactured and filled at [redacted] in [redacted] and the adjuvant CAF09b was manufactured and filled at [redacted] for SSI. The drug products and adjuvants are labelled at SSI according to good manufacturing practices (GMP). Saline (0.9% NaCl) for injection will be used as placebo, and water for injection will be used to reconstitute CTH522 for ID injection. Saline and water for injection will be ward stock, sourced commercially by the investigator site, in individual single-use ampoules.

The non-adjuvanted CTH522 to be given TO is ready to be used. The CTH522-CAF01 and CTH522-CAF09b (both to be given IM) and CTH522 to be given ID will be reconstituted at the site. The composition of the IMPs is described in Table 5.

Table 5: Composition of IMPs

IMP (routes)	CTH522	Per dose volume
CTH522-CAF01 (IM)	85 µg	0.5 ml
	15 µg	0.5 ml
CTH522-CAF09b[1] (IM)	85 µg	0.5 ml
CTH522 (TO)	24 µg[1]	0.04 ml
CTH522 (ID)	24 µg	0.1 ml

[1]subjects receive 12 µg in each eye.

9.2 Dose and administration of trial products

9.2.1 Dosage and route

Pre-clinical data from mice showed that there was a direct correlation between antigen dose and antibody titres, i.e. increasing the dose ten times (5–50 µg) lead to significant higher antibody titres and lowering the antigen dose (25–0.2 µg) lead to a drop in the antibody response. The lowest amount of CTH522 antigen needed to elicit a protective immune response in humans is unknown (please see Investigator's Brochure (IB) for more details). A literature review of recently published vaccine trials involving IM immunisation of recombinant proteins shows that well-tolerated doses range from 5–160 µg [14-19], and generally increasing the dose from 5–30 µg to 80–100 µg will increase the antibody titres [18, 19]. However, a further increase in dose does not seem to affect the antibody titre [15, 18]. Therefore, 85 µg CTH522 antigen with adjuvant $Al(OH)_3$ or CAF01 were tested in a non-clinical study in minipigs using IM and intranasal administrations. No treatment-related adverse clinical signs were recorded, and no evidence of systemic toxicity was found [9].

When comparing non-adjuvanted CTH522 with adjuvanted CTH522-$Al(OH)_3$ or CTH522-CAF01, a significantly higher level of anti-CTH522 IgG antibodies was measured by ELISA. Therefore, SSI evaluated a CTH522 antigen dose of 85 µg for IM immunisation in the first-in-human trial using IM and intranasal administration [9]. For the IM immunisations in the CHLM-01 trial, a classical prime-boost vaccination regime was adopted: a priming (two vaccinations four weeks apart)

followed by a boosting vaccination after 12 weeks. The CHLM-01 trial was designed to generate high titre primary antibody response following priming and allow enough time for the antibody affinity maturation process before boosting [20]. The results of that trial showed that a dose of 85 µg for IM immunisation was highly immunogenic when used in a classic prime-boost vaccination schedule [9].

For the CTH522 dose to be administered TO, a formulation containing 600 µg/ml CTH522 was found acceptable. Regarding the TO dose volume, a dose of 0.02 ml was deemed acceptable to avoid to significant spill-off from the topical ocular administration of a drop of CHT522. In combination, this consequently leads to a TO dose of 12 µg (given as 0.02 ml drop). In this trial, one drop is administered to each eye per vaccination, hence the total TO dose is 24 µg.

In the trial, it will be investigated if the TO or ID vaccination with CTH522 given concomitantly with CTH522-CAF01 IM will lead to increased immune responses. The TO dose of CTH522 is 24 µg, therefore the ID dose of CTH522 is also 24 µg, given as 100 µl ID.

The CAF01 adjuvant human dose was based on the results from the ACAF01-01 trial, a CAF01 dose-escalation trial completed in the Netherlands. The results of the ACAF01-01 trial concluded that a 625 µg/125 µg of DDA/TDB in CAF01 is the optimal human dose [12]. Also, this dose was found efficient when combined with CTH522 in the first-in-human clinical trial (CHLM-01) [9].

The CAF09b adjuvant is, like CAF01, a liposomic adjuvant formed by DDA and incorporating the MMG glycolipid into the liposome membrane for both stability and immunostimulatory reasons. The human dose level of CAF09b was therefore set based on the CAF01 dose levels of DDA and glycolid (TDB in CAF01 vs MMG in CAF09b), being 625 µg/125 µg of DDA/MMG in CAF09b. In addition to the DDA and MMG, the CAF09b contains the TLR3 agonist poly I:C, electrostatically bound to the liposome surface.

The poly I:C level had in pre-clinical studies (as the adjuvant CAF09) being at the same dose level as MMG. From a formulation and stability perspective, however, a lower dose of poly I:C relative to DDA/MMG was found optimal, resulting in 31 µg poly I:C combined with 625 µg/125 µg DDA/MMG to give CAF09b. This CAF09b 625/125/31 µg DDA/MMG/poly I:C was tested and found safe and acceptable in a repeated dose toxicological study in rabbits (as part of a toxicology study for a cancer vaccine) as well as the two ongoing clinical trials.

9.2.2 Treatment administered

The randomised subjects will be vaccinated with the IMPs (non-adjuvanted CTH522) and reconstituted IMPs (adjuvanted CTH522) accordingly to the schedule and route specified in Figure 1. On-site reconstitution of the adjuvanted IMPs is performed by mixing the CTH522 with CAF01 or CAF09b. Specific instruction of the on-site reconstitution can be found in the Vaccine Management Manual, which is prepared by SSI.

The preferred location for IM and ID administrations is into (IM) or into the skin overlying (ID) the non-dominant deltoid muscle. Each IM administration will consist of 85 µg CTH522-CAF01, 15 µg CTH522-CAF01 or 85 µg CTH522-CAF09b in a volume of 0.5 ml per injection. Each ID administration will consist of 24 µg CTH522 in a volume of 0.1 ml per injection. The IM and ID injections must be administered within five minutes of each other and approximately 2 cm apart from each other. As long as the two injections are administered within five minutes, it does not matter which one (IM or ID) is given first. The IM injection will be performed with a 1–2 ml polypropylene syringe via a 23–25-gauge needle. The ID injection will be with a 1 ml syringe via a microneedle device.

The TO administration in both eyes will consist of 24 µg CTH522 (non-adjuvanted) in a volume of 0.04 ml; thus, each eye is administered 12 µg of CTH522 TO in a 0.02 ml drop. The eye drops should be given five minutes apart, with the right side first to follow by the left side. The TO administrations will be performed using a Gilson MICROMAN positive displacement pipette, which will be provided by ICL.

Each colour coded vial and cardboard box of IMPs is labelled with the content of the vial as described in the Vaccine Management Manual. The identity of the injected trial vaccine will be known to the clinical site staff administering the trial vaccine and by the unblinded trial monitor. However, the identity of the trial vaccine administered will remain unknown to the subject during the trial.

The IMPs should only be administered by authorised staff as indicated in the investigational site signature and delegation form.

9.3 Packaging and labelling of trial products

The IMPs and adjuvants (excipients) are filled into vials (inner packaging) and are packed in cardboard boxes (outer packaging) according to GMP. The labels for inner- and outer packaging will be produced and printed at SSI according to the *'EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Annex 13, Investigational Medicinal Products'* [21]. Placebos are the commercially available saline solution.

9.4 Storage and shelf-life information for trial products

The IMPs and adjuvants must be stored according to the protocol in a safe and locked place at the site under the responsibility of the site PI and/or at central storage under the responsibility of a pharmacist. The expiry dates of the drug products and excipients are indicated on the labels of the cardboard boxes. They should not be used after the indicated dates. The final reconstitution of the IMPs and the storage conditions are described Vaccine Management Manual.

The freezers and refrigerators used to store IMPs and adjuvants must be equipped with thermometers, and the temperature must be controlled on a daily basis and documented in the storage condition logs under the responsibility of the PI/pharmacist. Relevant clinical site staff will

monitor the storage conditions of IMPs and adjuvants during the trial. In case of deviations in storage conditions identified by either the site investigator or monitor, SSI must be contacted to decide if they can be used in the trial or must be replaced.

9.5 Transport procedures for trial products

The drug products and adjuvants will be released by a qualified person, and subsequently transported to the clinical site when the clinical trial has been approved by the CA and by the relevant EC. The transport of the drug products and adjuvants will be arranged according to standard operating procedures at SSI. Temperature loggers will be used for monitoring the storage conditions during the transport. Procedures will be in place for documenting the dispatch of IMPs and adjuvants from SSI and the receipt at the site. During the trial, the monitor will check that the dispatch/receipt documentation in the investigator's file (IF) is adequate and correct.

9.6 Treatment compliance procedures

As this clinical trial is investigating vaccines that are administered by the staff at the site, the issue of subject treatment compliance will be addressed by evaluating the vaccine administration data recorded by the staff in trial-related documents/logs. During the trial, the monitor will check that vaccine administration data is recorded correctly.

9.7 Drug accountability procedures

It is the site's responsibility that all unused and used IMPs and adjuvants are accounted for during the trial and documented in the relevant trial documents/logs. Any unused IMPs and adjuvants are to be destructed after completion of the trial and after the approval by SSI. During the trial, the monitor will check that drug accountability is maintained. Empty vials and vaccine cardboard boxes are kept at the site until the close-out monitoring visit.

9.8 Precautions and overdosing

Anaphylactic reactions are very rare but a potential risk when administering vaccines. Therefore anaphylactic kit must be kept available for immediate use in the room where the injections are given. Medical staff trained in resuscitation must be in the clinic for the vaccination and 60 minutes after that.

Vasovagal syncope (i.e. fainting) can occur before, during and after injection and blood sampling. The site should minimise the occurrence of this reaction by allowing the subject to lie down or recline during the administration of vaccine and blood draws.

After administration of the vaccine, the subject must stay at the NIHR Imperial Clinical Research Facility (i.e. clinical site) for 60 minutes so that the staff can observe for any immediate AEs. Before the subject leaves the site, any AEs are reviewed and recorded in the source documents by delegated staff.

9.9 Concomitant medications

Non-trial vaccines received within 14 days before or after trial vaccination should be avoided. If a non-trial vaccine is to be administered, consider the following:

- Subjects should be advised (but not required) to increase the spacing between trial and COVID-19 vaccines to at least 28 days
- Non-trial vaccines should preferably be not injected into the same arm as the trial vaccine.

Systemic immunosuppressive agents, such as a corticosteroid, may not be administered during the trial. Dermal steroids are allowed but not if applied to the IM or ID injection sites.

Antipyretics/analgesics may not be taken within 24 hours before the day of vaccination. If taken, the vaccination should be postponed. Furthermore, during the first 72 hours after the vaccination, antipyretics/analgesics should be avoided. If taken, the details of the medication should be recorded in the diary card and in the CM pages of the eCRF.

Other medications (except for vitamins and minerals) are recorded in diary cards and subsequently transferred to the eCRF by relevant trial staff at the site.

10 Adverse events

The following definitions are in accordance with the EU Guidance (CT-3) (2011/C 172/01) and European Clinical Trial Directive 2001/20/EC of 4 April 2001 [22].

- An **AE** is any untoward medical occurrence in a subject administered a medicinal product and which does not necessarily have a causal relationship with the administered trial product. An AE can, therefore, be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medical product, whether or not considered related to the medicinal product. The definition also covers medication errors and uses outside what is foreseen in the protocol.

- An **adverse drug reaction** is an AE where a causal relationship to the trial product is at least a reasonable possibility, i.e. a causal relationship cannot be ruled out.

- An **unexpected adverse reaction** is a reaction that is by nature or severity of which is not consistent with the IB.

- An **unsolicited AE** is an event that will be sought from each vaccination, through non-leading AE questioning such as ‘how have you been feeling’, and through symptom-directed physical examination. Unscheduled laboratory testing and other investigations may be performed as required to investigate the AE.

- An **SAE or serious adverse reaction** is any untoward medical occurrence that at any dose:

  - results in death

  - is life-threatening. The term ‘life-threatening’ refers to an event where the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically could have caused death, had it been more severe

  - requires inpatient hospitalisation or prolongation of existing hospitalisation

  - results in persistent or significant disability/incapacity

  - is a congenital anomaly or birth defect

  - is an important medical event. The term refers to a medically important event that does not meet any of the SAE criteria but may require medical or surgical consultation or intervention to prevent one of the other serious outcomes listed above

- A **SUSAR** is a suspected unexpected serious adverse reaction according to the definitions given above.

10.1 Standard recording and assessment of adverse events

10.1.1 Collection and recording of AEs

The source of AEs covers, for example, the subject's response to questions about his/her health (a standard non-leading question), information from diary card, symptoms spontaneously reported by a subject, investigations and examinations where the findings are assessed by investigator to be clinically significant changes or abnormalities, other information relating to the subject's health becoming known to the investigator (for example hospitalisation).

Safety data are collected according to the timeframe specified in the flow chart (Table 3). The solicited AEs listed below will be collected via the diary cards:

- Solicited local injection site reactions after ID and/or IM administration of the vaccines: erythema, pruritus, pain, tenderness, swelling, and warmth
- Solicited ocular reactions after TO administration of the vaccine: watering eyes, swelling of eyelid, eye redness, and eye discomfort
- Solicited systemic reactions after IM and/or ID administration of the vaccines: oral temperature > 38.3°C, chills, myalgia and rash
- Any other AEs

The investigator or delegated staff must record all AEs, non-serious and serious, expected and unexpected in the relevant form of the eCRF.

The date of onset is the date when the first sign(s) or symptoms were first noted. If the AE is an abnormal clinically significant laboratory test or outcome of an examination the onset date is the date the sample was taken or the examination was performed.

If an AE or SAE is still ongoing or the outcome is unknown at the end of the trial (at the subject's last visit), it must be followed up by the investigator/delegate until it has been resolved or stabilised.

All SAEs must be recorded on the Serious Adverse Event form in the eCRF. If the investigator has unblinded the trial drug allocation of the subject, the screen-dump from the unblinding is forwarded together with the completed Serious Adverse Event (SAE) form to SSI (please see Section 5 for contact information).

10.1.2 Assessments

The investigator/delegate must assess the characteristics of the event(s) according to the below criteria:

- **Seriousness.** For the assessment of the seriousness of the adverse event, please see definition under Section 10.

- **Causality**. The causal relationship between an AE and the trial product must be assessed by the investigator using the terms listed below. A 'suspected adverse reaction' is defined as an AE which has been assessed as possibly, probably or certainly related to the product administered (i.e. which has a reasonable suspected causal relationship to the product administered):
    - Not related: An AE that is definitely NOT related to the product administered
    - Related: An AE that is either:
        - possibly related with a reasonable time relationship to product administration, but which can also be explained by concurrent disease, other drugs or other cause,
        - probably related with a reasonable time relationship to product administration, and which is unlikely to be attributed to concurrent disease, other drugs or other cause, or
        - certainly related with the AE occurring in a plausible time relationship to product administration and which cannot be explained by concurrent disease, other drugs or other cause.
- **Expectedness.** Expectedness is assessed according to the Reference Safety Information section in the newest version of the IB. No expected AEs are listed in the Reference Safety Information. Hence, all related SAEs in this trial should be reported as SUSARs.
- **Intensity.** The intensity of an AE must be assessed by the investigator using the FDA guidance in Appendix 1 [21]. Where a specific AE is not listed in the guidance, its intensity must be assessed by the investigator using the following terms:
    - Mild (Grade 1): No interference with daily activity
    - Moderate (Grade 2): Some interference with daily activity not requiring medical intervention
    - Severe (Grade 3): Prevents daily activity and requires medical intervention
    - Potentially life-threatening (Grade 4): Emergency visit or hospitalisation
- **Outcome.** The outcome of an AE must be assessed by the investigator using the following terms:
    - Recovered/resolved
    - Recovered/resolved with sequelae

- Recovering/resolving
- Not recovered/not resolved
- Fatal
- Unknown

10.2 Expedited reporting of adverse event

If an SAE occurs, immediate reporting is required by completing the Serious Adverse Event form, in the eCRF. The site PI must submit the completed SAE form via the eCRF at the latest within 24 hours of first knowledge. Once entered into the eCRF, the sponsor and delegated pharmacovigilance will be notified by email.

All SUSARs should be sent on an expedited basis by the CRO to the MHRA and the Research Ethics Committee according to the timelines listed below. The blinded SUSAR reports will be archived in the Trial Master File (TMF) at SSI and in the investigator´s file.

- For fatal or life-threatening SUSAR reports, notification as soon as possible, but no later than seven calendar days after first knowledge of the event and final reporting after an additional eight calendar days.
- For other SUSARs reporting, as soon as possible, but no later than 15 calendar days after first knowledge of the event.

A Development Safety Update Report should be prepared in accordance with ICH Guideline E2F on Development Safety Update Report at yearly intervals and submitted to the MHRA. The Development International Birth Date is 13-Mar-2016.

11 Data management and statistical analysis

11.1 General considerations

Data management and statistical analysis will be performed by [redacted] on behalf of SSI.

11.2 Data management

CHLM-02 is run as a Electronic Data Capture (EDC) trial, i.e. relevant data are entered by the site directly into the clinical database. Electronically transferred data will be handled and managed by [redacted] as well as the database and application.

The eCRF is designed to capture required information in compliance with GCP standards. The eCRF system is hosted by IBM eClinical, an EDC software and services company that is EU-US Privacy Shield certified.

All data, except for immunogenicity data, will be collected using an eCRF compliant with 21 CFR Part 11 regulation. Safety laboratory data will be collected via the local laboratories and transferred to the eCRF by the trial staff at the site.

Data management will be performed in accordance with applicable standards and data cleaning procedures. Only authorised access to the eCRF will be possible using an encrypted username and password. Roles in the system are given according to functions. All tasks performed in the eCRF are logged in an audit trail.

The eCRF will contain validation checks according to the Trial Validation Plan to maintain an ongoing quality check of data entered. All data validation will be performed as part of the system.

The investigator will approve the data using an electronic signature and thereby confirm the accuracy of the data recorded.

Medical History and AEs will be coded using the pre-defined version of the Medical Dictionary for Regulatory Activities (MedDRA) concerning system organ class, high-level group term, high-level term, preferred term and lowest level term. Concomitant medication will be coded using the WHOCC-ATC/DDD, an Anatomical Therapeutic Chemical classification system and the Defined Daily Dose drug dictionary. Coding of AE and CM shall be entered into the trial database.

Further details about trial setup and closure will be documented in the Data Handling Protocol prepared by [redacted].

11.3 Database release meeting and database lock

Before releasing the database for statistical analyses and trial reporting, the data manager will ensure that all quality control procedures in connection with data capture, cleaning and reconciliation of data have been finalised and documented. Protocol deviations will also be discussed at this meeting. The data manager is responsible for setting up a database release meeting with relevant trial team members. This meeting shall be held before the unblinding of the trial.

11.4 Statistical methods

The trial statistician prepares the SAS program/randomisation details description for the unblinded statistician/programmer. The unblinded statistician/programmer imports the randomisation list to the eCRF so that the site can automatically randomise the subjects via the eCRF and receive the allocated randomisation number and treatment.

The baseline is defined as the last assessment with available data before the first administration of trial medication.

The basis for safety analysis is the safety analysis set (SAF). The SAF consist of all subjects randomised and exposed to the IMPs, including subjects who are withdrawn after exposure to the IMP. The analysis of primary endpoints and all safety endpoints will be based on the SAF. Full

analysis set (FAS) will include subjects with observed data for all randomised subjects. A per-protocol analysis set (PP) will include subjects who completed the trial without deviations judged to influence the primary endpoints. The analysis sets will be defined at the data release meeting, which will take place prior to unblinding of the trial. The primary analysis will be carried out for SAF and FAS and PP.

Categorical data will be summarised descriptively by treatment, using the number and percentages of subjects. Continuous data will be presented using the number of subjects, mean, standard deviation, median, minimum and maximum. Both the absolute values and the change from baseline will be presented.

Primary efficacy data will be presented descriptively over time using box-plots over time. If the nature of data allow, a formal statistical test will be performed using time to event analysis.

11.4.1 Subject disposition

The disposition of subjects regarding inclusion in the SAF, FAS and PP and their trial completion status will be tabulated by treatment based on all randomised subjects and will also be presented in a listing. A separate listing of discontinued subjects will be included.

Protocol deviations will be summarised by treatment group for all randomised subjects.

11.4.2 Demographics and baseline characteristics

The three treatment groups (Cohort A-C) will be compared descriptively concerning a disposition, demography, baseline characteristics (age and race), medical history and CM, using both the SAF and the FAS. However, if the difference between SAF and FAS is two subjects or less, then only the SAF will be used. Similar comparison of the other cohorts.

11.4.3 Exposure and treatment compliance

It is planned that 91% of the subjects will randomise to receive the 85 µg CTH522-CAF01, 15 µg CTH522-CAF01, or 85 µg CTH522-CAF09b in addition to the successive administration of the non-adjuvanted CTH522 or the placebo. The other 9% of the randomised subjects will receive only the placebo consisting of 0.9% NaCl. Treatment compliance is documented as a drug accountability log.

11.4.4 Safety analysis

The binary primary endpoints are:

- Solicited injection site reactions after IM and ID administration with the following PTs: injection site erythema, injection site pruritus, injection site pain, injection site swelling and injection site warmth

- Solicited ocular reactions after TO administration with the following PTs: lacrimation increased, swelling of eyelid, ocular hyperaemia and ocular discomfort

- Solicited systemic reactions after vaccination with the following PTs: body temperature increased, chills, myalgia and rash

The frequencies of the binary endpoints will be presented with the corresponding 95% CI.

These endpoints will be analysed by pairwise comparison of active treatment groups versus placebo in 2 x 2 tables (presenting number and percentage of subjects experiencing the criteria) using Fishers exact test. Also, active treatment groups will be compared similarly.

Adverse drug reaction will be presented descriptively by treatment group (number of subjects, percentage of subjects experiencing the event and number of events), in tables of system organ class and PTs in summary tables presenting each group in separate columns.

Vital signs are presented descriptively by treatment group and visit. Both absolute values and changes from baseline are presented both in tables and in box-plots by visit.

11.4.5 Immunogenicity analysis

Percentage of subjects achieving seroconversion for anti-CTH522 IgG antibody at any time points after vaccination(s) will be descriptively presented. The criteria for seroconversion will be specified in the SAP by [redacted] Imperial and SSI. The SAP will be finalised before the unblinded review.

11.4.6 Exploratory immunogenicity analysis

Exploratory immunogenicity analyses will be performed separately from the primary and secondary analyses. Hence, the results from the exploratory analyses will not be included in the trial's CTR.

11.4.7 Interim analysis

No interim analysis is planned.

11.4.8 Sample size determinations

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.

12 Ethical aspects

The background of the CHLM-02 trial is to investigate the safety and immunogenicity of the chlamydia vaccine. SSI (sponsor) and ICL will, as part of the TracVac Consortium, conduct this trial and ICHT will host and act as the investigator site. The trial is partly funded by Horizon 2020. The grant covers expenses to materials, the staff at SSI and the investigator's site and the CROs managing data management, statistics and pharmacovigilance on behalf of SSI. Work items outsourced from SSI to ICL, investigator's site and the CROs are covered by signed agreements and Task Orders.

The trial will be conducted in accordance with the latest version of the Declaration of Helsinki [23] and ICH GCP. An application to conduct the trial will be submitted to MHRA and the EC for approval.

The subjects will receive verbal and written information about the purpose of trial and its nature, including details of any potential risks.

The total amount of blood required from each volunteer throughout the trial period of nine months will be approximately 900 ml as compared to 470 ml normally drawn from blood donors as a single donation; in the UK, men are allowed to donate every 12 weeks and every 16 weeks for women. Blood samples will be coded before the blood samples are sent for laboratory analyses. Only the investigator and his medical collaborators will have access to information that may link laboratory results with personal identification.

All non-clinical information supports the suitability of CTH522-CAF01 as a safe, immunogenic, and possibly effective vaccine to induce immunity in *C. trachomatis* uninfected individuals. The safety and reactogenicity of the CTH522 vaccines are expected to be comparable to the safety observed in a previous phase I clinical trial with CTH522 adjuvanted with CAF01 and $Al(OH)_3$ (CHLM-01) [9].

Based on animal toxicity studies [8] and the two ongoing clinical trials (ClinicalTrial.gov NCT03412786 and NCT03715985), CAF09b is expected to be safe and tolerable.

CTH522 adjuvanted with CAF09b has not been tested in humans before but based on the information above and the results of the non-human primates study (CTH522-CAF01, CTH522-CAF09b) and the EpiOcular *In vitro* test (CTH522) it is considered safe to initiate the present trial.

12.1 Risks and benefits

At present, there is only data available for CTH522 with or without the adjuvants CAF01 or $Al(OH)_3$ from a completed clinical trial that included 35 adult female volunteers [9]. From this trial, safety data where CTH522-CAF01 have been administered IM are known. The related AEs (> one in ten subjects) after IM administration of CTH522-CAF01 reported were:

- injection site pain, injection site tenderness and injection site movement impairment after IM vaccination
- sneezing, nasal congestion and rhinorrhoea after intranasal vaccination
- systemic reactions after IM or IN vaccination were headache, fatigue, malaise and myalgia

The most frequently reported treatment-emergent AEs were headache, oropharyngeal pain, and dysmenorrhoea. Please refer to the IB for more information.

Based on general considerations, experience from clinical trials with CAF01, CAF09b and CTH522, the IMPs to be used in the present trial are expected to be safe.

Anaphylactic reactions are very rare. However, appropriate medical treatment and trained staff will be available in case an anaphylactic reaction occurs.

Vasovagal syncope (i.e. fainting), may occur. It will be attempted to minimise the occurrence of this reaction, by allowing the subjects to lie down during the administration of vaccine and blood drawings.

The trial personnel performing the procedures will inform the subjects on the procedures before blood drawings and administration of vaccine, and it will be emphasised to the subject, that participation is voluntary and that he/she at any time can withdraw from the trial.

This is a healthy volunteer trial, so there will be no direct health benefit to the individual subject. However, they will undergo general health and sexually transmitted infection screening. Sexually transmitted infection may be asymptomatic, and if diagnosed, the subjects will be counselled and offered referral to the confidential service provided by their local NHS genitourinary medicine clinic. If trial results are satisfactory, further clinical trials will be initiated and may lead to a marketed vaccine against trachoma and/or genital chlamydia.

The subjects will receive payment to compensate them for their time, inconvenience, travel expenses, etc. They will be paid £100 per scheduled clinic visit, to a maximum of £1200, paid as a lump sum at the end of participation. Volunteers who attend screening but who are not enrolled will not receive payment.

12.2 Biobanking

The storage, handling and use of the samples in the SSI Biobank are in compliance with relevant personal data protection laws and EC regulations.

After the finalisation of the clinical trial, the serum samples will be stored at the research biobank at SSI and ICHT. The samples may be used for standardisation, quality control and future assays related to chlamydia vaccine research and development. The samples will only be identifiable by a

subject number. The subject will be informed of this procedure in the information sheet, and consent will be sought.

13 Good clinical practice considerations

13.1 Declaration of Helsinki and good clinical practice

The clinical trial is planned, and will be conducted and reported in compliance with this protocol, as well as the latest versions of the 'Declaration of Helsinki' [23], the ICH E6 (R2) [24] and Directive 2001/20/EC [25].

13.2 Participant information and informed consent

The written subject information and the informed consent form (ICF) will not be taken into use until approved by the EC(s) and UK Health Research Authority (HRA).

The subjects will be recruited through the clinical site's healthy volunteer database, posters and information meetings at universities and other public forums, advertisements in newspapers and social media. All written recruiting material will be approved in advance by the concerned EC.

Before the first trial-related visit, the potential subject are given ample time to reflect on the trial information. At the first visit, the subject information and ICF are reviewed thoroughly with the subject. The verbal review will always be conducted by a physician or nurse, and encompasses:

- Comprehensible presentation of background and objectives of the clinical trial
- Presentation of the trial design (randomised)
- Any predictable benefits, risks, adverse reactions, inconveniences, complications and disadvantages
- Potential risks
- Right to reflection
- Right to waive information on clinical trial results
- Description of the IMPs
- That collection and extradition of information to/from the subject's subject files in the Health Care System may occur
- Remuneration and reimbursement of transportation expenses
- Questions from the subject

The subjects will always be given the opportunity to discuss participation with a physician prior to first dosing.

It is emphasised to the subject that participation is voluntary and that he/she at any time can withdraw from the trial. The potential subject is informed that data collected during participation in the trial is confidential.

The review of the written information with the subject and the signing of the ICF take place under undisturbed environment. Should there be a need for further reflection before signing the consent form, the potential subject is offered another 24 hours to reflect. A new appointment is made for the signing of the ICF. Once the ICF is signed, medical examination and other trial-related activities can be performed.

13.3 Ethics committee and HRA submission and approval

The trial will NOT be initiated until it has been approved by both EC and HRA. The PI and the sponsor will collaborate on the submission of the project to the EC and HRA. An electronic initial submission form is completed on www.myresearchproject.org.uk. The PI and the sponsor must sign the submission form and send to the EC together with the other required documentation.

13.4 Competent authority submission and approval

A request for a clinical trial authorisation will be submitted for approval to the MHRA in the UK. The application will be submitted by ICL on behalf of SSI as GCP sponsor of the trial.

13.5 Local investigator site approval

Screening or inclusion of subjects will only be initiated after approval by the clinical site's local NHS R&D Office, which is issued only after EC, HRA and MHRA approval.

13.6 Personal data protection

The subjects' personal data will be protected in accordance with the EU General Data Protection Regulation and the Clinical and Directive 2001/20/EC. The PI is responsible for keeping a screening log of trial subjects including personal information such as full name, address and personal identification number.

The trial documents containing personal information, e.g. the screening log and the signed ICFs, will be kept in a safe place at the investigational site at all times, and will not be transferred to the sponsor or any third party for 25 years.

All other trial documents or electronic records will not contain personal information but will identify the trial subjects by use of a subject number. Only, through linking this subject number to the screening log, the PI can identify the subject.

At the completion of the trial, normally at the site-closure monitoring visit, the site PI must date and sign off the subject log(s) for completeness.

13.7 Investigator's responsibility and delegation of trial-related duties

The site PI is responsible for:

- The conduct of the clinical trial in accordance with the protocol, current GCP guidelines, and other applicable national requirements and regulations
- Documenting the receipt, storage, dispensing and accountability of drug products and adjuvants as appropriate
- All data including AEs being recorded in the eCRFs and for the immediate reporting of SAEs to SSI and CRO as described in Section 10
- Being available to answer or clarify any protocol related queries from the CA or ECs
- Keeping a log of all subjects
- Signing the Investigator Statement Form

If the site PI delegates her responsibilities to other staff members, it has to be documented in the clinical site signature and delegation form. This documentation has to be completed before trial initiation, which is to be updated during the trial and to be signed off for correctness at the end of the trial. The PI is responsible for supervising any individual or party to whom she delegates trial-related duties.

Before trial initiation, SSI must collect current, dated and signed curriculum vitae for all participating investigators and other relevant trial staff including monitors and data managers. The signature and delegation form(s) and the dated and signed curriculum vitae must be kept in the IF.

13.8 Trial oversight and monitoring

The sponsor will conduct trial oversight by employing a risk-based approach and other quality control measures. Trial oversight activities are described in the Trial Oversight Manual.

Monitoring will be performed by SSI according to SSI GCP standard operating procedures and a trial specific monitoring plan. The purpose of the monitoring is to verify the rights and well-being of the trial subjects, the accuracy, completeness and correctness of the data and the conduct of the trial according to this protocol, GCP, applicable trial manuals and applicable regulations and laws. Qualifications of the monitor(s) will be kept in the TMF at the sponsor's site.

A trial initiation monitoring visit will be performed at the site before enrolment of the first subject in the trial, and subsequent routine monitoring visits will be performed during the trial as per the monitoring plan. At trial completion, a close-out monitoring visit will be performed.

13.9 Audit and inspection

The selected site for the present trial participated in the previous CTH522 trial, CHLM-01. Before the CHLM-01 trial, the site was audited by the Quality Assurance staff from SSI. No further audit for the current trial is planned. The audit documentation from CHLM-01 will be filed in this trial's TMF. Shall there be a need for additional audit or inspection, the site must give access to the auditors and inspectors to all trial-related documents, including the subject identification log(s) and/or personal medical records. The PI would need to inform the sponsor immediately and vice versa, if an inspection has been requested by an authority.

13.10 Source data and essential documents and retention times

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations or other activities in the trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

At the site, a document, which identifies all (expected) source documents and the location of these, will be prepared (and signed off by the PI) before the initiation of the trial. Examples of clinical site sources are:

- EC and CA approval documents
- Signed ICFs
- Subject screening log/identification code list
- Investigator's or nurse's notes/worksheets (for data not recorded or not recorded directly in the eCRF)
- eCRF for data recorded directly in the eCRF
- Completed diaries
- Completed CIOMS reporting forms (generated by Pharmacovigilance)
- Vaccine inventory and accountability log(s)
- Serum sample inventory and accountability log(s)
- Logs of monitoring of storage conditions of IMPs and serum samples

It is the responsibility of the PI to archive all source data (all source documents from which the eCRF entries are derived) in the trial subject's medical records according to the national recommendation.

Entries in the eCRF will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The trial monitor from SSI will check the eCRF for accuracy and completion and perform source data verification according to the trial monitoring plan.

Essential documents are those documents which individually or collectively permit evaluation of the conduct of the trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor (via monitoring) with the standards of GCP.

Before initiation of the trial, an IF will be set up at the investigational site and a TMF at the sponsor's site following the ICH E6 (R2) guideline. These systems will contain essential documents. The completeness of the IF with the GCP essential documents will be documented as part of the site-closure monitoring visit. The IF and the TMF shall retain the essential documents for the timeframe stipulated in their respective national laws.

14 Agreement and financial settlement

An agreement between SSI (trial sponsor) and ICHT (investigator site) will be signed before inclusion of the first subject. These agreements will clearly state the rights and obligations of the concerned parties and are the legally binding documents between the parties. This protocol, in its current version, or its current version at any time in case of subsequent modifications (amendments), will be an appendix to the agreement between the investigator institution and the sponsor. The agreement must be signed by the legal representatives of the parties before the inclusion of the first subject in the trial.

ICHT must grant the PI permission to conduct the trial on its premises. This permission must be given before the inclusion of the first subject in the trial.

This trial is funded by a grant from the EU Framework Programme for Research and Innovation Horizon 2020. The results of the trial will be published according to the dissemination strategy as detailed in the TracVac Consortium Agreement.

15 Insurance and indemnity statement

SSI carries a product liability insurance programme including cover for clinical trials. The insurance programme covers worldwide and is currently placed with insurer XL Insurance (Appendix 2). The policy covers claims arising from injury/injuries caused by trial medication used in this clinical trial sponsored by SSI, if the trial product has been used in accordance with the instructions given in the

protocol. An updated insurance certificate will be provided separately, without updating the protocol, if applicable.

In the event that any recruited subject in the trial should suffer any personal injury resulting from participation in the clinical trial, SSI agrees to indemnify the institution where the clinical trial is being undertaken, and through the institution, any of its employees or agents participating in the trial, against liability imposed by law, but not assumed voluntarily, and arising from the use of the trial products, provided that:

- SSI shall not indemnify against, nor have any obligation whatsoever as regards liability arising from or related to any error, omission, intentional wrongful act, or other negligence on the part of said institutions or persons, such as medical malpractice
- any such institution or person seeking indemnity
- has fully complied with the protocol for the trial
- has promptly notified SSI of any notice of any type of claim, or the likelihood of a claim, relating to the trial
- as regards any claim, makes no statement, takes no action, nor makes any commitment affecting SSI's interests, without SSI's prior written consent, and further, provides all reasonable and necessary assistance to SSI in the defence of any claim, allowing SSI, at its cost and in its discretion to take over the defence of any action and to have full control in handling the claim

Before initiation of the clinical trial, SSI will present an indemnity statement to the PI signed by the director of Vaccine Development Department.

16 Confidentiality and disclosure

All eCRF data, information and results generated by SSI, as well as information on product development, patented or not, including patent applications and manufacturing processes not previously published, are considered confidential and shall remain the sole property of SSI.

No data from the clinical trial, unless approved by SSI in writing, may be published, presented or communicated, except to MHRA or EC(s), before the issue of the final CTR or before being published. A CTR will be prepared by SSI. The names of authors and their order of appearance in the publication are stated in the agreement.

17 Modifications to the protocol

The trial procedures may be modified if the signing parties agree to the modifications. If the modifications are substantial, MHRA, HRA, EC and ICHT R&D must be notified as required, and approve the modifications before implementation. All substantial modifications should be documented by issuing new amended versions of the protocol implementing the modifications.

18 References

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19 Appendices

19.1 Appendix 1: FDA guidelines

Guidance for Industry

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact the Division of Vaccines and Related Products Applications, Office of Vaccines Research and Review at 301-827-3070.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
September 2007

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Guidance for Industry

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

Preventive vaccines are usually developed to prevent disease in a healthy population. The Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, regulates preventive vaccines under authority of section 351 of the Public Health Service Act (42 U.S.C. 262), as well as specific sections of the Federal Food, Drug, and Cosmetic Act, and reviews investigational new drug applications (INDs) and biologics license applications (BLAs). (See, for example, Title 21 Code of Federal Regulations (CFR) Parts 312, 600, and 601). Most of the clinical trials of preventive vaccines conducted to support INDs and BLAs enroll healthy volunteers in all phases of vaccine testing. The enrollment of healthy volunteers warrants a very low tolerance for risk in those clinical trials.

This guidance provides you, sponsors, monitors, and investigators of vaccine trials, with recommendations on assessing the severity of clinical and laboratory abnormalities in healthy adult and adolescent volunteers enrolled in clinical trials. The grading system described in the table can also be useful in defining a particular study's stopping rules (e.g., a certain number of adverse events, as defined in the table, may call for stopping the study). Less extreme observations (e.g., mild) may not require discontinuing the study vaccine but can still contribute to evaluating safety by identifying parameters to focus upon in subsequent product development. Uniform criteria for categorizing toxicities in healthy volunteers can improve comparisons of safety data among groups within the same study and also between different studies. We, FDA, recommend using toxicity grading scale tables, provided below, as a guideline for selecting the assessment criteria to be used in a clinical trial of a preventive vaccine. We recommend incorporation of such appropriate, uniform, criteria into the investigational plan, case report forms, and study reports and correspondence with FDA, sponsors, monitors, investigators, and IRBs.

This guidance finalizes the draft guidance of the same title dated April 2005 (70 FR 22664, May 2, 2005).

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FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Standardized toxicity assessment scales have been widely used to evaluate products treating specific diseases. For example, the National Cancer Institute's Common Toxicity Criteria Scale and the Division of AIDS' Toxicity Grading Scale standardize the evaluation of adverse events among patients with cancer and HIV/AIDS, respectively (Refs. 1, 2). The defined toxicity parameters in those scales are designed for patients who may already experience mild, moderate, or severe adverse clinical or laboratory events due to the disease process, and may not be appropriate for healthy volunteers.

In the development of the toxicity grading scales for healthy volunteers, we chose parameter limit values based on published information, when such values were available (Refs. 1-6). For example, the Brighton Collaboration has developed case definitions and guidelines to evaluate some adverse events associated with administering vaccines (Ref. 3). In some cases, parameter limit values were based on clinical experience and experience reviewing vaccine clinical trials that enroll normal healthy subjects.

Toxicity grading scales for laboratory abnormalities should consider the local laboratory reference values when the parameter limit values are defined. The characterization of laboratory parameters among some populations of healthy adults and adolescents may require the exercise of clinical judgment, for example, consideration of the potential for ethnic differences in white blood cell (WBC) counts or gender differences in creatine phosphokinase (CPK) values.

III. TOXICITY GRADING SCALE TABLES

Adverse events in a clinical trial of an investigational vaccine must be recorded and monitored and, when appropriate, reported to FDA and others involved in an investigation (sponsors, IRBs, and investigators). (See, for example, 21 CFR 312.32, 312.33, 312.50, 312.55, 312.56, 312.60, 312.62, 312.64, 312.66). Although the use of a toxicity grading scale for adverse events would not replace these regulatory requirements, using a scale to categorize adverse events observed during a clinical trial may assist you in monitoring safety and making required reports. Nonetheless, we believe that categorization or grading of data as outlined in this document is supplementary to and should not replace full and complete data analysis.

These guidelines for toxicity grading scales are primarily intended for healthy adult and adolescent volunteers. The parameters in the tables below are not necessarily applicable to every clinical trial of healthy volunteers. The parameters monitored should be appropriate for the specific study vaccine. For some preventive vaccines under development, it may be appropriate

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to include additional parameters to be monitored during a clinical trial or to alter the choice of values in the toxicity table. For example, additional parameters might be added based on one or more of the following: safety signals observed in pre-clinical toxicology studies, the biological plausibility of the occurrence of certain adverse events, or previous experience with a similar licensed product.

As discussed above, the tables do not represent a recommendation to monitor all the listed parameters in all clinical trials of healthy volunteers, nor do the tables represent all possible parameters to be monitored. In addition, these tables do not represent study inclusion or exclusion criteria. We recommend that the parameters monitored be appropriate for the study vaccine administered to healthy volunteers participating in the clinical trial.

A. Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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Vital Signs *	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

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Systemic Illnes	Mild (Grade 1)	(Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

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B. Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***ULN" is the upper limit of the normal range.

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Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** "ULN" is the upper limit of the normal range.

Urine *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

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IV. REFERENCES

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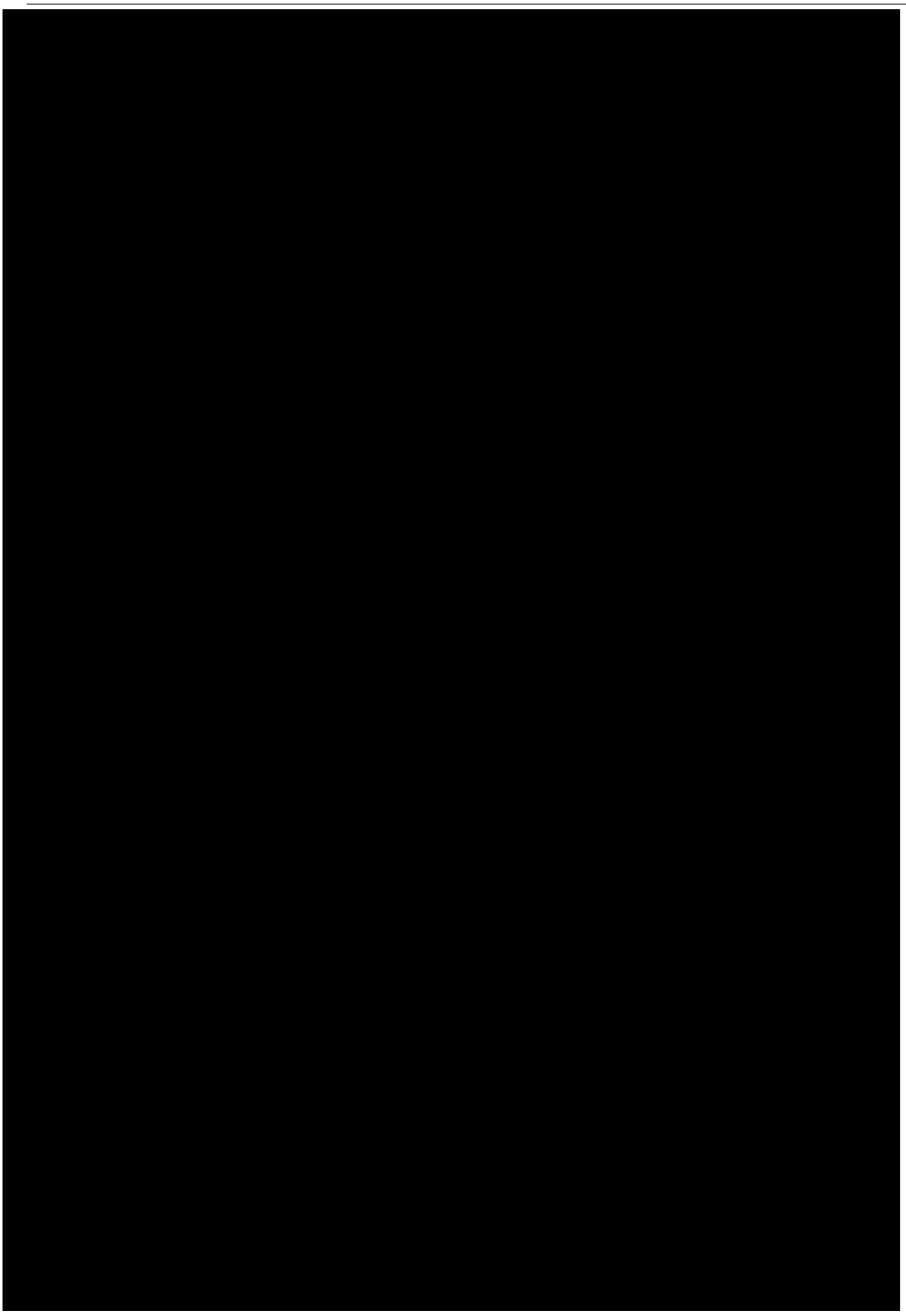
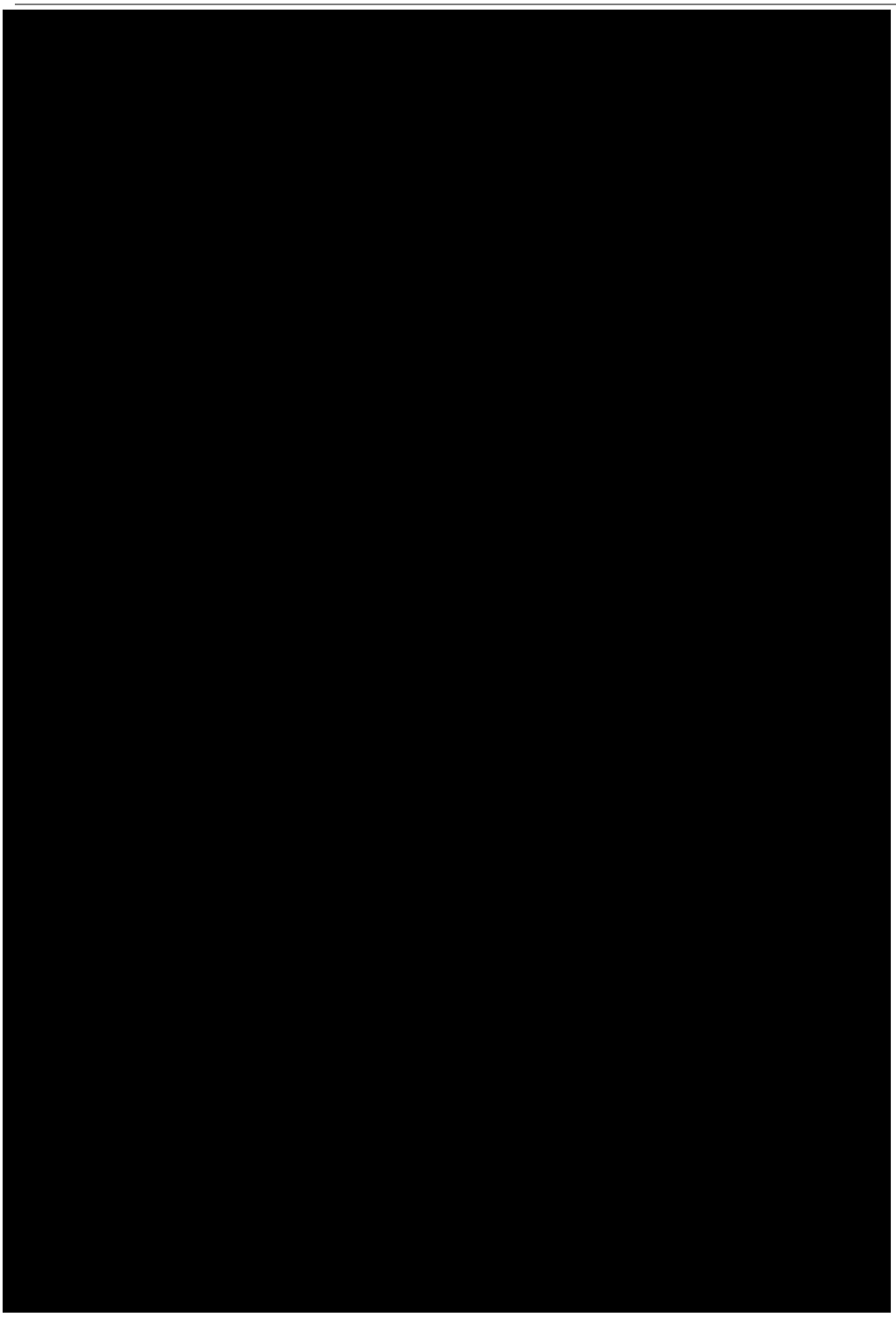
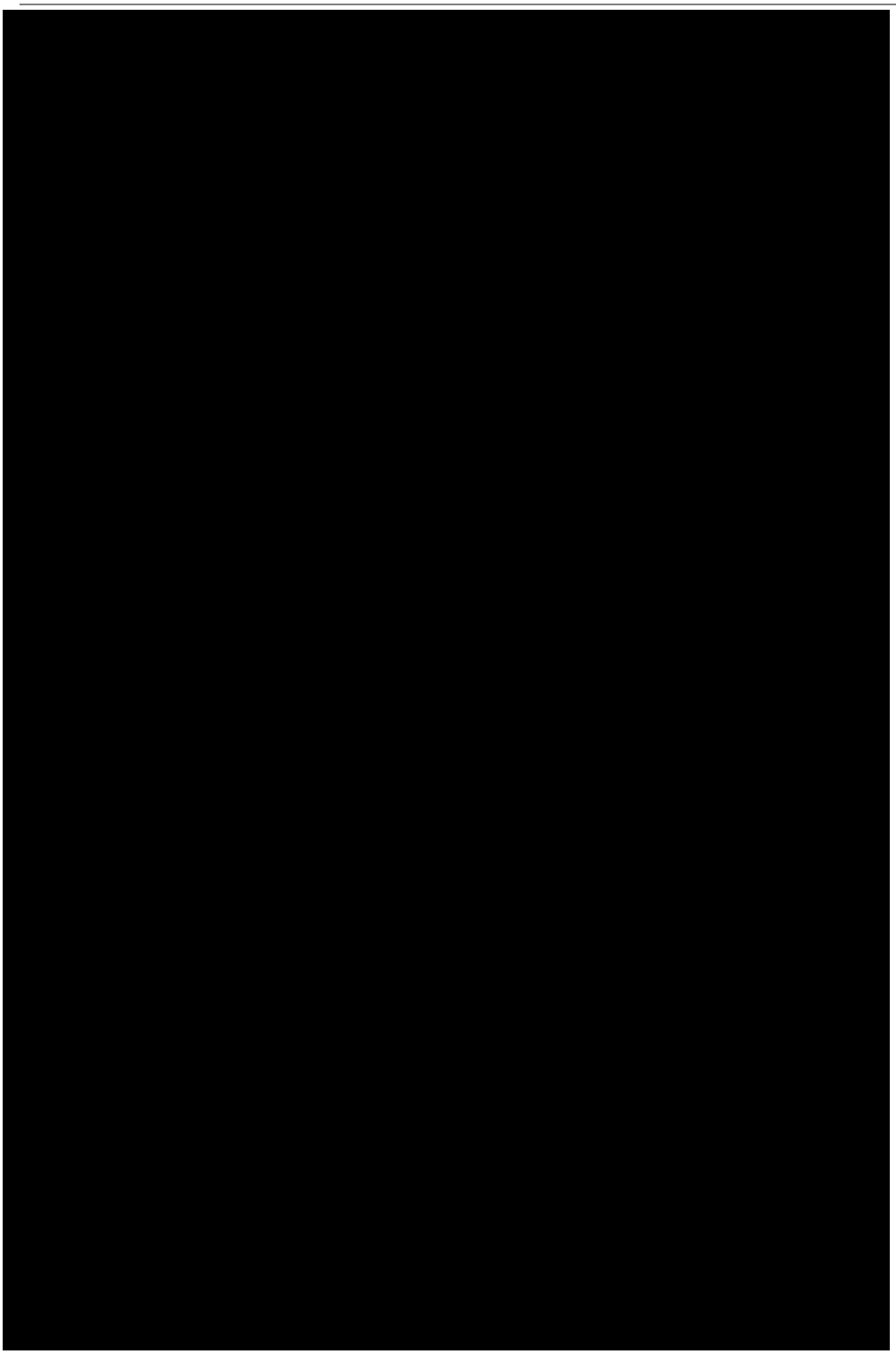
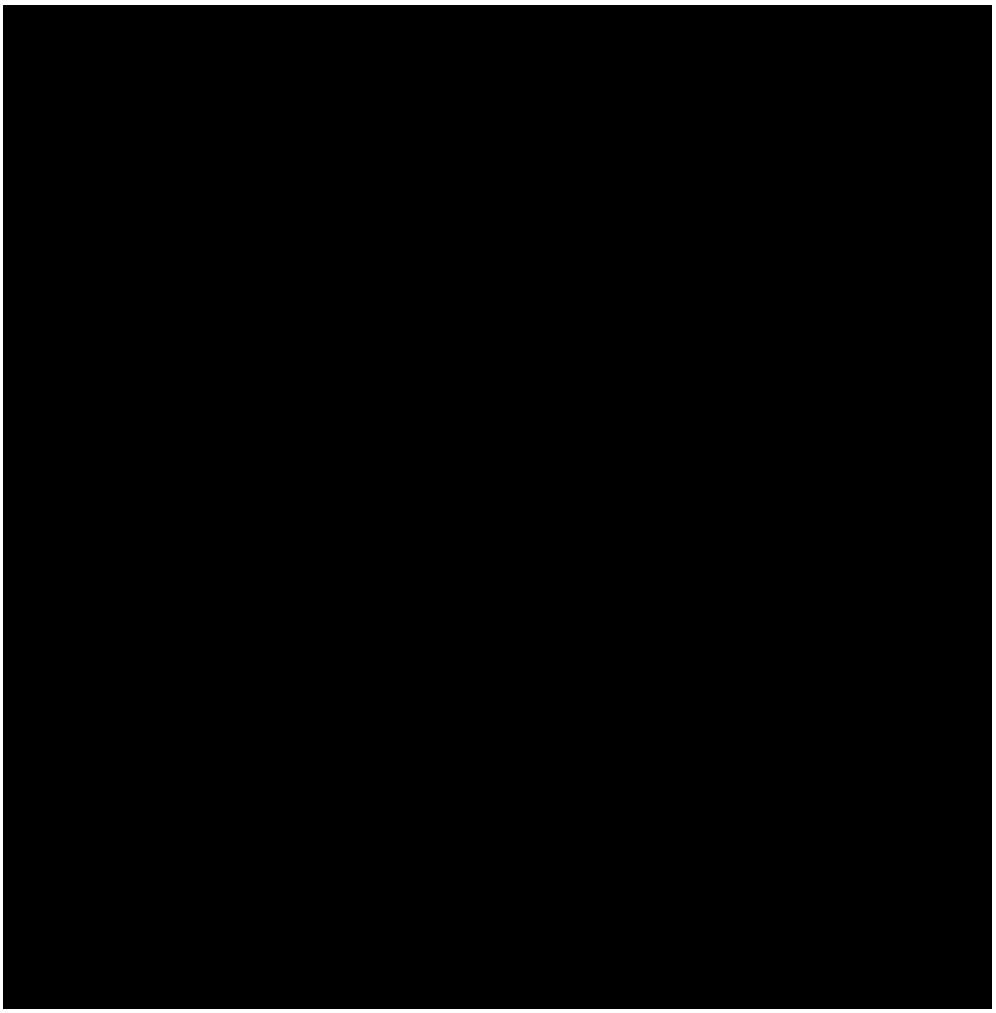
Material Transfer Agreement (hereinafter referred to as the "MTA")

[redacted]









19.2 Appendix 2: Insurance certificate