

Title: A phase I double-blind trial investigating trachoma vaccine regimens using the Chlamydia vaccine CTH522 administered with cationic liposomes in healthy adults (CHLM-02)

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Summary

Background

There is no vaccine against the major global pathogen *Chlamydia trachomatis*, its' different serovars causing trachoma in the eye or chlamydia in the genital tract. In this clinical trial, CTH522 a recombinant version of the *C. trachomatis* major outer membrane molecule, was given in different dose levels with and without adjuvant, to determine its' safety and immunogenicity when administered intramuscularly (IM), intradermally (ID) and topically into the eye (TO), in prime-boost regimens.

Methods

CHLM-02 was a phase I, double-blind, randomised, placebo-controlled trial at the National Institute for Health Research Imperial Clinical Research Facility, London, UK. Participants were healthy men and non-pregnant women aged 18 to 45 years, without pre-existing *C. trachomatis* genital infection. Participants were assigned into six arms by the electronic database in a pre-prepared randomisation list (A-F). Investigators were masked to treatment allocation. Arms A-E received investigational medicinal product (IMP) and arm F received placebo only. Two liposomal adjuvants were compared, CAF01 and CAF[®]09b. At Day 0, all participants received an IM priming

injection; arms A-C received 85µg CTH522-CAF01, arm D received 15µg CTH522-CAF01 and arm E received 85µg CTH522-CAF[®]09b. At Day 28 and Day 112, two booster doses followed, participants receiving the same IM injection as the priming dose plus either ID or TO CTH522 or placebo. The final TO dose at Day 140 was 24µg CTH522 or placebo. The objectives were to evaluate the safety of, and the humoral and cellular immune responses engendered by, the different formulations, dose levels and routes of administration of CTH522. Analyses were conducted as intention to treat and as per protocol.

Findings

Between Feb 17, 2020 and Feb 22, 2022, 65 participants were randomised, and 60 completed the trial (52% [34/65] women, 71% [46/65] white, mean age 26.8 years). No serious adverse events (AEs) occurred but one participant discontinued dosing after experiencing self-limiting AEs after both placebo and IMP doses. Study procedures were otherwise well tolerated; the majority of AEs were mild to moderate, with only 7/865(0.8%) reported as grade 3 (severe). There was 100% fourfold seroconversion rate by day 42 in the active arms and no seroconversion in the placebo arm. Serum IgG anti-CTH522 titres trended higher after 85µg CTH522-CAF[®]01 than 15µg (p=0.062), with no difference after three injections of 85µg CTH522-CAF[®]01 compared with CTH522-CAF[®]09b. ID CTH522 (arm C) induced high titres of serum IgG anti-CTH522 neutralising antibodies against serovars B (trachoma) and D (urogenital). TO CTH522 (arm B) at Day 28 and 112 induced higher total ocular IgA compared with baseline (p<0.001). Participants in all active vaccine arms, particularly arms B and E, developed cell mediated immune responses against CTH522.

Interpretation

CTH522, with CAF[®]01 or CAF[®]09b, is safe and immunogenic, with 85µg CTH522-CAF[®]01 inducing robust serum IgG binding titres. ID vaccination conferred systemic IgG neutralization breadth, and TO administration increased ocular IgA formation. These findings indicate CTH522 vaccine regimens against ocular trachoma and urogenital chlamydia for testing in Phase 2 clinical trials.

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Research in context

-Evidence before this study: We searched PubMed using the terms “chlamydia vaccine” or “trachoma vaccine” and “clinical trial”, with no restrictions on publication dates or language, and identified one reported chlamydia vaccine trial (London, UK, 2019) and six references to clinical trials of trachoma vaccine undertaken in Taiwan, Saudi Arabia, Northern India and Ethiopia published between 1966 and 1979. The present study is the second clinical trial of a vaccine with immunogenicity against trachoma and urogenital chlamydia serovars, and the first to assess ocular immune responses of a vaccine against *Chlamydia trachomatis* since the trachoma trials in the 20th century.

-Added value of this study: We deployed an innovative trial design to accelerate the clinical development of the CTH522 chlamydia vaccine by comparing the safety and immunogenicity of two different dose levels, two different adjuvants and three boosting approaches; intramuscular (IM) with adjuvant, and intradermal (ID) or topical ocular (TO) without liposomal adjuvant. IM CTH522, adjuvanted with either CAF® 01 or CAF® 09b, was safe and immunogenic. Systemic IgG immune responses were enhanced by boosting with ID unadjuvanted CTH522. Ocular IgA responses were enhanced and sustained throughout the study (Day 238) by two TO unadjuvanted CTH522 doses. These findings are important for the design of future chlamydia vaccine trials to determine whether these responses correlate with protection from ocular trachoma and urogenital chlamydia.

-Implications of all the available evidence: The promising safety and immunogenicity profile of the vaccine regimens encourages clinical development of vaccines for both trachoma (with a TO boost) and urogenital chlamydia (with an ID boost). If further testing indicates that these routes protect against disease, this would support investment in their clinical delivery to maximise ease of administration for mass vaccination campaigns.

Manuscript body text

Introduction

C. trachomatis is the aetiologic agent of a variety of clinical syndromes in humans depending on the route of infection and causative serovar. Serovars D-K cause urogenital *C. trachomatis* infection and disease, serovars A-C are related to ocular trachoma, and serovars L1-L3 are related to lymphogranuloma venereum. Infected epithelial cells in mucosal surfaces serve as key innate immunity responders and drivers of the immune response, but long-term morbidity is associated with chronic inflammation and fibrotic tissue damage.¹

Trachoma is a neglected tropical disease affecting impoverished populations with inadequate access to sanitation.² 136.9 million people live in trachoma-endemic areas, 1.9 million of whom are visually impaired or blind due to trachoma. While the global burden of trachoma has significantly decreased due to improvements in sanitation and mass drug administration, the prevalence of trachoma remains high in some populations. In fact, 44 countries (26 of which in Sub-Saharan Africa) still require interventions against trachoma.³ The development of an effective vaccine would be of paramount importance to accelerate progress towards trachoma control.

The vaccine antigen CTH522 is a recombinant version of the major outer membrane protein (MOMP) from *C. trachomatis*, designed to induce broadly neutralising antibodies against *C. trachomatis* infection. CTH522 is highly immunogenic and contains conserved T cell epitopes frequently recognised by *C. trachomatis* infected persons, combined with a neutralising antibody response against urogenital *C. trachomatis* serovars D-G and K plus ocular serovars A and B.⁴ In a first-in-human phase I randomised trial with 35 women,⁵ we assessed CTH522 administered either with the liposome-based cationic adjuvant formulation CAF[®]01, which was designed to promote strong neutralising antibody titres and Th1 responses,⁶ or aluminium hydroxide (AH). Both vaccines were safe and immunogenic, and CTH522-CAF[®]01 showed accelerated seroconversion, higher IgG titres, enhanced mucosal antibody profile, and more consistent cell-mediated immune response compared with CTH522-AH.⁵

In the present trial we assessed the safety and immunogenicity of different novel vaccine regimens to generate both systemic and ocular immunity. We investigated intramuscular administration of the liposomal adjuvants CAF[®]01, developed to provide immune signaling through C-type lectin Mincle receptor and CAF[®]09b that also contains a toll-like receptor (TLR) 3 agonist.⁷ For induction of ocular mucosal responses, we investigated administration of non-adjuvanted CTH522

administered by topical ocular (TO) or intradermal (ID) route, both together with CTH522-CAF[®]01 IM. After completion of the vaccination regimen, all arms were examined for the magnitude of recalled immunity induced by a mucosal boost with TO CTH522.

Methods

Study design

CHLM-02 was a phase I, double-blind, parallel, randomised, and placebo-controlled trial done at the Imperial Clinical Research Facility at Hammersmith Hospital in London, UK. The study protocol was approved by London-Chelsea Research Ethics (19/SC/0353) and The Medicines and Healthcare products Regulatory Agency (MHRA) (EudraCT No.: 2019-001090-88). The trial was conducted in accordance with the principles of Declaration of Helsinki and the International Conference on Harmonisation's (ICH) Good Clinical Practices (GCP) and is registered with ClinicalTrials.gov (NCT03926728). The study protocol is available as a supplemental appendix.

Participants

We enrolled healthy male and female participants between 18–45 years old. Participants of child bearing potential had a negative pregnancy test and agreed to use acceptable contraceptive measures during the trial. Trial participants had BMI < 35 kg/m², no significant history of cardiac, liver, immunologic, neurologic, psychiatric disease, no clinically significant abnormality of haematological or biochemical parameters, negative blood tests for HIV, hepatitis B/C, syphilis, and negative urine PCR tests for *C. trachomatis* and gonorrhea. Screened participants were excluded if they were participating in another clinical trial, had received a vaccine within 14 days of the start of the trial, received systemic immunosuppressive agents, or had allergy to any of the vaccine constituents. Participants were also excluded if they had a medical history of or an active ocular disease, and if they were unable to refrain from use of contact lenses during TO administration. Women breastfeeding and/or with a confirmed history of pelvic inflammatory disease or significant gynaecological diseases were excluded.

We recruited participants via the Imperial Clinical Research Facility's healthy volunteers' database, posters, and advertisements at local university and NHS sites and on social media. All trial participants gave written informed consent before entering the trial.

Randomisation and masking

We randomly assigned trial participants into 12 arms (Figure 1). Arm A received three IM vaccination of 85µg CTH522-CAF® 01. This arm was further divided into two arms: A1 received ID placebo at day 28 and 112, and TO placebo at day 140, while A2 received TO placebo at day 28 and 112, and unadjuvanted TO CTH522 mucosal recall (12 µg in each eye) at day 140.

Arm B received three IM vaccinations of 85µg CTH522-CAF® 01. This arm was further divided into two arms: B1 received TO vaccination of the unadjuvanted CTH522 at day 28 and 112 and TO placebo at day 140, while B2 received the same for day 28 and 112, and unadjuvanted TO CTH522 at day 140. The two additional TO doses of CTH522 (12 µg) were administered in each eye.

Arm C received three IM vaccinations of 85µg CTH522-CAF® 01. This arm was further divided into two arms: C1 received ID vaccination of the unadjuvanted 24µg CTH522 at day 28 and 112 and TO placebo at day 140, while C2 received the same for day 28 and 112, but TO 12 µg CTH522 mucosal recall in each eye at day 140. Arm D received three IM vaccinations of 15µg CTH522-CAF® 01. Arm E received three IM vaccinations of 85µg CTH522-CAF® 09b. Arm F received placebo in form of sodium chloride 0.9% (IM, ID and TO). To keep the trial blinded TO and ID placebo were administered as in arm A.

The main purpose of including TO and ID administrations in addition to the IM was to test if these vaccination routes demonstrated a higher ability to facilitate an ocular immune response. Our goal was to elicit robust ocular immune responses through two regimens: 1) manipulation of the systemic immunity (using different adjuvants and with concomitant ID administration); and 2) by a mucosal TO CTH522 administration in combination with CTH522-CAF® 01 administered intramuscularly. A TO administration was included, since the anatomical ocular site may require direct assess of the vaccine antigen to the ocular mucosa, to facilitate a local IgA response.⁸ ID vaccination has been extensively studied, and the dermis and/or epidermis of human skin has been shown to be an efficient site for antigen delivery, priming of immune responses and dose-sparing.^{9,10} To compare

vaccine adjuvants for their capacities to elicit functional immune memory we employed a non-adjuvanted antigen recall TO.¹¹ The late TO recall response was tested in all active arms through a TO administration at day 140 with either unadjuvanted CTH522 or placebo.

Randomisation was performed using a randomly-generated sequence of numbers managed by a validated module in the electronic case report form (eCRF). The randomization list was prepared by a blinded statistician. Randomisation data were kept confidential, accessible to authorised unblinded persons, until the time of unblinding. Masking was done by shielding the participants from seeing the preparation of the investigational medical product (IMP). The trial personnel preparing and administering the IMP were not involved in any trial assessments (safety or immunogenicity). During trial drug administration, a blinded staff member, also shielded from seeing trial drug or any related procedures, was present. This blinded staff member observed the subject and monitored any adverse events (AEs) during or after trial drug administration. The codes were broken and made available for data analysis upon database lock.

Procedures

The IMPs were produced under good manufacturing practice by the Statens Serum Institut (SSI), Copenhagen, Denmark. The drug products CTH522 (for IM, ID and TO uses) and adjuvant CAF® 01 were manufactured and filled at Baccinex SA in Switzerland, and the adjuvant CAF® 09b was manufactured and filled at AJ Vaccines in Denmark for SSI. Sodium chloride 0.9% for injection was used as placebo, and water for injection reconstituted lyophilised CTH522 for ID injection. The non-adjuvanted CTH522 administered TO was manufactured ready to be used. The CTH522-CAF® 01 and CTH522-CAF® 09b administered IM and CTH522 administered ID were reconstituted at site. The composition of each IMP used in this trial is described in supplemental table 1 (Appendix, page 2).

Trial arms and dose regimens are shown in Figure 1. The preferred location for IM and ID administrations was into the non-dominant deltoid muscle (IM) or into the skin overlying it (ID). The IM and ID injections were administered within five minutes of each other and approximately two cm apart from each other. The eye drops were given five minutes apart, with the right side

first followed by the left side. ID injections were administered with the NanoPass microneedle device, and TO injections with a Gilson MICROMAN® positive displacement pipette.

Safety was evaluated via participant diaries and questioning by investigators, safety laboratory analysis, eye examinations, physical examinations, and vital sign assessments. Eye examinations were conducted at every visit. Fundoscopy could not be conducted during COVID-19 due to NHS Trust restrictions on close personal contact. Biomicroscopy via slit lamp examination was conducted by specialists at the Western Eye Hospital, London, UK at the first visit and at three follow-up visits (day 42, 126 and 154). Blood sampling for safety was performed at all study visits and covered full blood count, liver function tests and renal profile results. Abnormal and clinically significant laboratory results were reported in the eCRF as adverse events (AE). Participants were monitored after each vaccination for immediate AEs after 60 (+/- 10) minutes. Participants were also asked if they had experienced AEs since the last visit. From each vaccination until the visit scheduled 14 days later, specific AEs (injection site reactions, ocular reactions, and systemic reactions) were solicited through diary cards.

Blood samples for quantification of anti-CTH522 IgG and IgA levels with ELISA were collected at baseline and 14, 28, 42, 56, 112, 126, 140, 154, and 238 days afterwards. Ocular IgG and IgA anti-CTH522 were quantified in ocular strips with ELISA. Ocular strips were collected at baseline and 28, 42, 56, 126, 140, 143, 154, and 238 days afterwards. Peripheral blood mononuclear cells were collected at the same intervals to assess cell mediated immune responses with interferon- γ (IFN- γ), interleukin-17 (IL-17), and memory B cell enzyme-linked immune spot (ELISpot), activation-induced markers (AIM), and flow cytometry for intracellular cytokine staining (ICS) of CD4⁺ and CD8⁺ T cells. The cell-mediated immune response assays (ELISA and ELISpot),^{5,12} as well as the assay to measure neutralising antibodies against serovars B and D,¹³ have been described elsewhere. The AIM and ICS assays are described in the appendix, pages 17-18. .

Outcomes

The co-primary safety outcomes were: a) solicited local injection site reactions after ID and/or IM administration of the vaccines (erythema, pruritus, pain, tenderness, swelling, and warmth), b) solicited local reactions after TO administration of the vaccine (watering eyes, swelling of eyelid,

eye redness, and eye discomfort), c) solicited systemic reactions after IM and/or ID administration of the vaccines (oral temperature > 38.3°C, chills, myalgia and rash), and d) any other adverse events (AEs).

The secondary outcome (humoral immunogenicity) was the percentage of trial participants achieving anti-CTH522 IgG seroconversion, defined as four-fold and ten-fold increase over baseline levels. Exploratory outcomes included systemic levels of CTH522-specific IgG, ocular levels of CTH522-specific IgG and IgA antibodies, serum neutralising antibodies against serovars B and D, and cell-mediated CTH522-specific immune responses.

Statistical analyses

This was a hypothesis-generating trial with an innovative design. As such, there was no power to detect significant differences between study arms. The sample size of 66 participants was considered adequate to assess the safety of IMPs in a phase 1 trial.

Categorical data were summarised by study arm, using number and percentages of participants. For calculation of percentages, the denominator was the number of participants in the analysis set (intention to treat or per protocol). To assess the primary outcome of safety, the percentage of study participants with AEs was compared between study arms using the Chi-square test. 95% CIs for the proportion of participants experiencing side effects were calculated using the Clopper-Pearson method.

Continuous data were presented using the number of trial participants (n), with means and standard deviations (SD) if normally distributed, or with medians and interquartile ranges in case of a skewed distribution. Anti-CTH522 IgG titre values were compared between the arms using non-parametric Mann-Whitney-Wilcoxon exact two-sample test. We also conducted a post hoc analysis of anti-CTH522 IgG titre at day 28 to assess whether levels of vaccine-induced antibodies varied by gender. This analysis was performed on log transformed titre using analysis of covariance (ANCOVA) including fixed, categorical effects of treatment, gender, and treatment-by-

gender interaction and log transformed baseline titre as covariate. The log-normal assumption was confirmed by assessment of the normality and homogeneity of variance of the residuals.

Ocular IgG and IgA levels after vaccination were compared to baseline levels using Kruskal-Wallis with Dunn's multiple correction test. Neutralising antibodies after vaccination were compared to baseline by Mann-Whitney-Wilcoxon exact two-sample test (day 0 versus day 126). Correlations of neutralising antibodies and serum IgG anti-CTH522 were assessed with the Spearman's rank correlation coefficient.

Cell-mediated immune responses after vaccination were compared to baseline level using Kruskal-Wallis test with Dunn's multiple correction test or Mann-Whitney-Wilcoxon exact two-sample test. The p-values were not adjusted for multiple comparisons.

Role of funding source

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Results

Between Feb 17, 2020, and Feb 22, 2022, a total of 154 subjects were screened. Enrollment of trial participants was delayed due to lockdown measures in response to the COVID-19 pandemic. Due to the approaching expiry date of IMPs, enrolment was stopped after 65 participants had been randomised. Reasons for exclusion are listed in supplemental Figure 1 (Appendix, page 9). 60 participants completed the trial. Five randomised participants withdrew from the trial at their own request; they were equally distributed across arms.

All 65 randomised participants were included in the intention to treat analysis. A total of 37 participants were included in the per protocol analysis set. Besides the five trial withdrawals, 23

participants with 29 major protocol deviations were excluded from per protocol analyses (supplemental Table 2 [Appendix, page 3]). Two participants were excluded from PP due to TO administration difficulties. One participant was excluded due to intradermal administration difficulties.

The trial population (Table 1) comprised 34 women (52%, 34/65) and 31 men (48%, 31/65); 71% (46/65) were white, with a mean age (standard deviation) of 26.8 years (6.8). Not all arms were well-balanced for gender due to small number of participants enrolled in each arm. Arms E1 and F2 comprised only female participants and arm A1 comprised male participants only.

Injection site reactions were reported by nearly all participants in the active arms after the first parenteral vaccination, and most participants after the second and third vaccinations (Table 2). The most frequently reported injection site reaction was pain. The only significant difference found was among trial participants in arms A-C (85 µg IM CTH522-CAF® 01), who had significantly more solicited injection site reactions (98%, 58 out of 59 participants) than placebo (33%, 2 out of 6) after the first vaccination ($p=0.0007$) (supplemental Table 3 [Appendix, page 5]). No differences between arms A-C (85 µg IM CTH522-CAF® 01) and arm D (15 µg IM CTH522-CAF® 01) or between arms A-C and arm E (85 µg IM CTH522-CAF® 09) were found.

Participants in most arms reported ocular reactions after the active or placebo TO. However, no differences were found when comparing the arms receiving active TO (arms B1 and B2) with the group receiving TO placebo (arm A2) or with the placebo arm (F1).

Solicited systemic reactions were reported in all active arms but not in the placebo arms. The most common systemic reaction was myalgia. No difference was found in the number of participants reporting systemic reactions after the first vaccination when comparing the pooled arms receiving the 85µg of CTH522 adjuvanted with CAF® 01 (arms A-C) with either the 15µg dose (D), or with the arms receiving CTH522 adjuvanted with CAF® 09 (E) or with the placebo arm (F) (supplemental Tables 4-6 [Appendix, pages 6-8]).

No serious adverse events or deaths occurred during the trial. No participants were withdrawn from the trial due to adverse events, but one participant vaccinated with CTH522-CAF® 01 and TO placebo reported vomiting and headache after the second IM dose on day 26. The site investigator

attributed both events as probably related to the IMPs and permanently discontinued IM vaccinations. The subject only received TO vaccination (placebo) on day 105 and reported three mild adverse events (headache, vomiting, and watery eyes), all with onset on the dosing day. The participant was discontinued from all dosings and fully recovered from AEs.

CTH522 IgG seroconversion, defined as either four-fold or ten-fold increase of IgG anti-CTH522 over baseline levels, was observed among all trial participants receiving active vaccine. Seroconversions occurred after the second parenteral vaccination and were sustained throughout the study period (Figure 2).

Although not statistically significant, anti-CTH522 IgG titres were five-six-fold higher among participants randomised to arms with 85µg CTH522-CAF® 01 than with 15µg CTH522-CAF® 01 at day 28 ([intention to treat: median IgG titre ratio arms A-C/D=5.6, p=0.062] [per protocol analysis: arms A-C/D =6.4; p=0.176]). No significant differences in terms of IgG titres were observed between participants receiving 85µg CTH522 adjuvanted with either CAF® 01 (arms A-C) or CAF® 09 (arms E).

ID CTH522 led to higher anti-CTH522 IgG titres than TO CTH522 at day 126. Participants randomised to arms C (85µg IM CTH522-CAF® 01 + ID CTH522) had higher levels of anti-CTH522 IgG than those randomised to arms A (85µg IM CTH522-CAF® 01 + placebo [intention to treat: median IgG titre ratio arms A/C=0.2; p=0.099] [per protocol analysis: median A/C=0.1; p=0.024]) or arms B (85µg IM CTH522-CAF® 01 + TO CTH522; [intention to treat: median IgG titre ratio arms B/C=0.3, p=0.056] [per protocol analysis: median B/C=0.2; p=0.016]).

We found no evidence for a significant difference between men and women in terms of anti-CTH522 IgG levels (P-value for gender interaction in IgG levels at day 28= 0.243).

Memory B cell ELISpot (supplemental Figure 2) was conducted at baseline, after the third parenteral vaccination (day 140), after mucosal recall with TO CTH522 (day 154), and the final study visit (day 238). Significant memory B cell ELISpot responses were observed in all active vaccine arms. Responses peaked on days 140 and 154 but returned to baseline levels on day 238. Memory B cell ELISpot responses in the blood did not seem to be affected by the mucosal antigen recall on day 140.

Ocular anti-CTH522 IgG levels were significantly higher than baseline following vaccinations in all active vaccine arms, except for arm A. We found no noticeable differences in ocular IgG between study arms receiving TO (arm B) or ID CTH522 (arm C) boosts, and the effect of each boosting regimen, if any, seems to be comparable (supplemental Figure 3).

Conversely, ocular anti-CTH522 IgA levels were significantly higher than baseline following TO vaccinations for arm B from day 126 (two weeks post-third vaccination [$p=0.005$]) onwards. Ocular IgA levels were consistently higher following TO CTH522 administration in arm B when compared to all other arms (Figure 3).

There was no further increase in ocular IgG and IgA levels with a mucosal antigen recall with TO CTH522 on day 140 in any arm.

Overall, results of intention to treat and per protocol analyses were consistent (data not shown)

Neutralising antibodies against ocular (serovar B) and genital serovars (serovar D) were induced in all active vaccination arms on day 126 when compared to baseline. Despite the trial's limited power for direct comparisons between study arms, median titres of neutralising antibodies against serovar B and serovar D were numerically higher after ID CTH522 (arms C) than after TO CTH522 (arms B) in the full analysis set (Figure 4), pointing out a stronger effect of ID CTH522 on systemic humoral immune responses.

Neutralising antibodies levels against serovar B were strongly correlated with serum anti-CTH522 IgG (Spearman $r = 0.729$, 95% CI = 0.567 - 0.837, $p<0.0001$) and with neutralising antibodies against serovar D on day 126 (Spearman $r= 0.849$, 95% CI = 0.747 - 0.911, $p<0.0001$). Neutralising antibodies levels against serovar D were strongly correlated with serum IgG anti-CTH 522 levels on day 126 (Spearman $r= 0.832$, 95% CI = 0.721 - 0.902, $p<0.0001$).

Cell-mediated immune responses were assessed by IFN- γ and IL-17 ELISpot conducted at baseline, after the third parenteral vaccination (day 126), after mucosal recall with TO CTH522 (day 154), and the final study visit (day 238).

Significantly higher IFN- γ ELISpot responses were observed in nearly all active vaccine arms post-vaccination when compared to baseline (supplemental Figure 4). Responses peaked on day 126

and remained significantly higher than baseline until day 238. Responses were not increased by the mucosal antigen recall TO dose on day 140. IFN- γ ELISpot responses were not significantly higher than baseline levels in arm C. This could be the result of a small sample size or a larger variability of responses at baseline in arm C compared to other arms.

IL-17 ELISpot responses post vaccination were less prominent than IFN- γ responses (supplemental Figure 5) and were significantly higher than baseline in arms A and C only. IL-17 ELISpot responses also tended to peak on day 126 but returned to baseline levels afterwards.

Flow cytometry with intracellular cytokine staining (ICS) to assess CD4⁺, and AIM T cell responses to the CTH522 protein were assessed at baseline and after the third parenteral vaccination (day 126).

The percentage of CD4⁺ cells with combined responses of IFN- γ , TNF- α and IL-2 (ICS assay) on day 126 was significantly higher than baseline in arms A, B and E (supplemental Figure 6).

T cell responses (AIM assay) on day 126 were significantly higher than baseline in nearly all active vaccine arms, except arms A and E, where higher variability of responses at baseline and small sample size may have hampered our ability to detect significant differences in immune responses (supplemental Figure 7).

Overall, although no formal inter-arm comparisons were made, we conclude that there were no noticeable differences of cell-mediated immune responses between arms vaccinated with 15 μ g and 85 μ g CTH522, adjuvanted with CAF[®] 01 and CAF[®] 09, or with ID and TO CTH522. Cell-mediated immune responses were not increased by the mucosal antigen recall TO dose on day 140.

In general, with respect to cell-mediated immune response, results of intention to treat and per protocol analyses were consistent (data not shown).

Discussion

We deployed an innovative trial design to accelerate the clinical development pathway of a chlamydia vaccine by comparing, head-to-head, two different vaccine antigen doses, two different

adjuvants and two unadjuvanted vaccine boosting approaches. IM CTH522, adjuvanted with either CAF® 01 or CAF® 09b, was safe and immunogenic. 85µg CTH522 was more immunogenic than 15µg, as demonstrated by higher serum IgG anti-CTH522 titres at day 28. Higher cell-mediated immune responses and significant memory B cell ELISpot responses were observed in all active vaccine arms compared to placebo. The present study is the second clinical trial of a vaccine with immunogenicity against trachoma and urogenital chlamydia serovars, and the first to assess ocular immune responses of a vaccine against *Chlamydia trachomatis* since the trachoma trials in the 20th century.¹⁴⁻¹⁹

The study procedures were well tolerated. However, one participant was withdrawn from dosing after experiencing adverse events. Given that these events, headache and vomiting, were observed after both active IMP administered IM and placebo administered TO on separate occasions, it is not possible to attribute this directly to CTH522 from these data. Occurrence of these adverse events should be monitored for in future larger studies with a placebo-controlled design to determine causality.

No correlate of protection against chlamydia infection and disease exists to guide clinical development. The prevailing view is that a future vaccine should generate antibodies as well as T cell responses.²⁰ In addition, since *C. trachomatis* serovars infect via mucosal routes, protective local immunity may be required of the vaccine to protect against either infection or disease. Here we tested vaccine regimens to generate both systemic and ocular immunity.

Systemic humoral immune responses, involving both binding IgG and neutralising antibodies, were enhanced by giving unadjuvanted CTH522 as a booster shot, intradermally. CMI and memory B cell responses were unchanged. ID delivery has historically been used to enhance the immunogenicity of vaccines and for antigen dose sparing.^{9,10} Given its immunogenicity, intramuscular priming followed by intradermal boosting with CTH522 holds promise as a vaccination strategy against urogenital chlamydia, provided that the systemic responses we measured can be validated as correlates of protection against chlamydia infection and/or disease in future studies.²¹

Ocular mucosal immune responses in the form of ocular anti-CTH522 IgA were enhanced by unadjuvanted CTH522 given as a booster dose directly into the eye. This booster vaccine was well

tolerated. A post hoc analysis of serum IgA levels suggested that the ocular IgA was mucosally-derived and not merely a transudate from the serum (supplemental Figure 8). Therefore, this vaccination strategy, i.e. TO boosting of a parenteral vaccination, holds promise for future testing in efficacy trials for infections that require ocular IgA for protection.

The ability to induce mucosal IgA responses has been linked to the CAF® 01 adjuvant immune-profile²² and is in agreement with results from a previous clinical trial of CTH522/CAF® 01, in which an intranasal boost facilitated IgA responses in vaginal and nasal mucosae.⁵ This suggests that induction of secretory ocular IgA requires direct access of the vaccine antigen to ocular draining lymphoid tissue. Ocular IgA has neutralising and anti-inflammatory properties, which could protect against trachoma and avoid excessive inflammation and immune-mediated pathology.²³

There are similarities among the distinct *C. trachomatis* disease syndromes in terms of protective immunity and immunopathology. Despite being designed to cover urogenital serovars D-G, CTH522 also induced neutralising antibodies against ocular serovar B. The CTH522 vaccine molecule also contains large segments of MOMP shared among both ocular and genital tract isolates, and these segments are known to contain both shared B and T cell epitopes.²⁴ It is thus enticing to hypothesise that CTH522 can offer cross-protection against different serovars and clinical syndromes. As a proof of concept, cross-protection of meningococcal vaccines against gonorrhoea has been reported in register-based epidemiologic studies.²⁵ In a recent randomised trial, vaccination with a meningococcal B vaccine (Bexsero®) halved the risk of incident gonorrhoea in men who have sex with men.²⁶

Our study has limitations. First, sample size was small lacking power to detect significant differences between study arms. We cannot conclude on which is the most immunogenic and best tolerated adjuvant; the lack of difference between CAF® 01 and CAF® 09b arms could be attributable to the small sample size. Subject withdrawals may have led to random confounding and selection biases. Secondly, our trial was conducted during the COVID-19 pandemic, with a substantial number of protocol deviations. Trial staff experienced difficulties when administering the ocular CTH522 with the Gilson MICROMAN® pipette, which is not a standard method of eye drop administration, but only a few cases were actually excluded from the per protocol analyses

due to this. Future development of ocular vaccination should include a standardised method of delivery, which is robust to differences in operator skillset.

To conclude, IM CTH522, adjuvanted with either CAF® 01 or CAF® 09b, holds promise for future testing in efficacy trials against urogenital *C. trachomatis* and trachoma. An ID boosting regimen coupled with parenteral vaccination may be particularly suitable for urogenital infection provided that the higher humoral responses we observed following ID CTH522 correlate with protection against infection at the urogenital mucosa in future studies. A TO boosting during or after parenteral priming represents a promising vaccine regimen against trachoma, due to induction of local ocular IgA. Whether CTH522 should be further supplemented to enhance protection against all trachoma serovars remains to be tested.

Contributors

RS, JD and FF conceived the study. HMC, IR and RES performed the statistical analyses. KMP was the chief investigator. AHB was the study director and drafted the manuscript. HMC designed the CMI experiments and analysed the results. IDR designed the neutralisation assay experiments and analysed the results. MPK qualified and did the serum IgG and IgA ELISA analyses. All authors contributed to data interpretation, critically revised the manuscript and approved the final version.

Declaration of interests

KMP had membership of the data safety monitoring board for NCT05249829 and has membership for NCT05575492, has received a fee for speaking from Seqirus and Sanofi Pasteur, and has research funding from the Chan Zuckerberg Initiative, the MRC/UKRI, the Vaccine Task Force, and NIHR Imperial BRC outside the submitted work. PA, FF, IR and AO are co-inventors on a patent on vaccines against chlamydia [US10925954, EP2976355]. All rights have been assigned to Statens Serum Institut, a Danish not-for-profit institute under the Ministry of Health. The authors have no other relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Other authors have no conflicts of interest to declare.

Data sharing

Data including de-identified participant safety and immunogenicity data along with study documents including the protocol and informed consent forms may be shared with other investigators upon written request to the corresponding author.

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Intention to treat	A1 CTH-01 ID-PCB TO-PCB	A2 CTH-01 TO-PCB TO	B1 CTH-01 TO TO-PCB	B2 CTH-01 TO	C1 CTH-01 ID TO-PCB	C2 CTH-01 ID TO	D1 CTH-01 _{low} TO-PCB	D2 CTH-01 _{low} ID-PCB TO	E1 CTH-09 TO-PCB	E2 CTH-09 ID-PCB TO	F1 Placebo TO-PCB	F2 Pla ID- TO
Intention to treat set (N,%)	2 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	3
Gender (N,%)												
Female		5 (56)	1 (33)	1 (11)	1 (33)	6 (67)	2 (67)	5 (56)	3 (100)	5 (56)	2 (67)	3
Male	2 (100)	4 (44)	2 (67)	8 (89)	2 (67)	3 (33)	1 (33)	4 (44)		4 (44)	1 (33)	
Ethnicity (N,%)												
White	1 (50)	6 (67)	3 (100)	4 (44)	3 (100)	6 (67)	3 (100)	6 (67)	1 (33)	7 (78)	3 (100)	3
Asian		1 (11)		1 (11)		3 (33)		1 (11)	1 (33)	1 (11)		
Black or African American		1 (11)						1 (11)		1 (11)		
Other	1 (50)	1 (11)		4 (44)				1 (11)	1 (33)			
Age (years)												
Mean (SD)	22·0 (14)	26·9 (6·4)	21·7 (2·1)	28·4 (7·3)	32·3 (6·1)	22·4 (2·4)	25·7 (9·0)	26·8 (5·6)	31·7 (8·5)	30·1 (9·9)	24·7 (5·5)	25
Min - Max	21 - 23	19 - 36	20 - 24	22 - 45	27 - 39	19 - 25	20 - 36	20 - 35	23 - 40	19 - 45	19 - 30	21
Height (cm)												
Mean (SD)	181·0 (12·7)	173·7 (7·5)	181·7 (10·1)	176·1 (8·7)	178·3 (6·7)	171·8 (12·7)	171·0 (11·1)	165·2 (7·2)	167·3 (1·5)	171·8 (4·3)	177·0 (6·2)	16
Min - Max	172 - 190	164 - 184	170 - 188	162 - 190	174 - 186	151 - 191	161 - 183	156 - 177	166 - 169	165 - 178	172 - 184	15
Weight (kg)												
Mean (SD)	69·0 (22·6)	74·9 (11·0)	78·0 (4·4)	70·2 (12·8)	83·3 (8·1)	70·0 (12·1)	72·3 (9·3)	63·3 (7·8)	60·7 (1·5)	71·4 (12·7)	76·7 (14·2)	64
Min - Max	53 - 85	55 - 88	75 - 83	55 - 91	74 - 88	54 - 91	66 - 83	48 - 74	59 - 62	56 - 92	64 - 92	57
BMI (kg/m ²)												
Mean (SD)	20·7 (4·0)	24·8 (3·0)	23·7 (2·3)	22·6 (3·6)	26·2 (2·6)	23·6 (2·4)	24·7 (1·6)	23·1 (1·7)	21·7 (0·9)	24·1 (3·6)	24·4 (3·2)	22
Min - Max	17·9 - 23·5	20·3 - 29·8	21·5 - 26·0	16·9 - 29·0	24·2 - 29·1	18·9 - 27·2	23·1 - 26·2	19·5 - 24·7	20·7 - 22·5	20·1 - 30·7	20·9 - 27·2	22

Table 1: CHLM-02 baseline characteristics

IM: Intramuscular, ID: Intradermal, TO: Topical ocular, PCB: Placebo

Cohort A1: 85 µg IM CTH522-CAF01 + ID Placebo + TO Placebo, Cohort A2: 85 µg IM CTH522-CAF01 + TO Placebo + TO CTH522, Cohort B1: 85 µg IM CTH522-CAF01 + TO CTH522 + TO Placebo, Cohort B2: 85 µg IM CTH522-CAF01 + TO CTH522
Cohort C1: 85 µg IM CTH522-CAF01 + ID CTH522 + TO placebo, Cohort C2: 85 µg IM CTH522-CAF01 + ID CTH522 + TO CTH522, Cohort D1: 15 µg IM CTH522-CAF01 + TO Placebo, Cohort D2: 15 µg IM CTH522-CAF01 + ID Placebo + TO CTH522
Cohort E1: 85 µg IM CTH522-CAF09b + TO Placebo, Cohort E2: 85 µg IM CTH522-CAF09b + ID Placebo + TO CTH522,
Cohort F1: IM Placebo + TO Placebo, Cohort F2: IM Placebo + ID Placebo + TO Placebo

CHLM-02 co-primary outcomes (safety)	A* CTH-01 N (%)		B* CTH-01 N (%)		C* CTH-01 N (%)		D* CTH-01 _{low} N (%)		E* CTH-09 N (%)		F* Placebo N (%)		Active IM total N (%)	Placebo total N (%)
Intention to treat (N, %)	11 (100)		12 (100)		12 (100)		12 (100)		12 (100)		6 (100)		59 (100)	6 (100)
Any solicited local injection site reactions after IM vaccination (day 0 + 14 days)	10 (91)		12 (100)		12 (100)		12 (100)		12 (100)		2 (33)			
Injection site pain	10 (91)		12 (100)		11 (92)		12 (100)		12 (100)		1 (17)		57 (97)	1 (17)
Injection site warmth	1 (9)		5 (42)		5 (42)		2 (17)		3 (25)		1 (17)		16 (27)	1 (17)
Injection site swelling	1 (9)				1 (8)		1 (8)		3 (25)				6 (10)	
Injection site erythema	1 (9)				1 (8)				2 (17)				4 (7)	
Injection site pruritus					3 (25)				1 (8)				4 (7)	
Any solicited systemic reactions after IM vaccination (day 0 + 14 days)	2 (18)		3 (25)		3 (25)		3 (25)		2 (17)				13 (22)	
Myalgia	1 (9)		1 (8)		3 (25)		2 (17)		2 (17)				9 (15)	
Chills	1 (9)		3 (25)				2 (17)						6 (10)	
Pyrexia			2 (17)										2 (3)	
Rash			1 (8)				1 (8)						2 (3)	
CHLM-02 co-primary outcomes (safety)	A1 CTH-01 ID-PCB TO-PCB N (%) E	A2 CTH-01 TO-PCB TO N (%) E	B1 CTH-01 TO TO-PCB N (%) E	B2 CTH-01 TO N (%) E	C1 CTH-01 ID TO-PCB N (%) E	C2 CTH-01 TO N (%) E	D1 CTH-01 _{low} TO-PCB N (%) E	D2 CTH-01 _{low} ID-PCB TO N (%) E	E1 CTH-09 TO-PCB N (%) E	E2 CTH-09 ID-PCB TO N (%) E	F1 Placebo TO-PCB N (%) E	F2 Placebo ID-PCB TO-PCB N (%) E	Active IM - total N (%) E	Placebo - total N (%) E
Safety analysis set (N, %)	2 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	9 (100)	3 (100)	3 (100)	59 (100)	6 (100)
Any solicited local injection site reactions after ID and/or IM vaccination (day 112 + 14 days)	1 (50) 3	6 (75) 12	2 (67) 5	8 (89) 20	3 (100) 10	8 (89) 33	1 (50) 3	7 (78) 16	2 (67) 5	8 (100) 26	2 (67) 5	1 (33) 1	46 (78) 133	3 (50) 6

Injection site pain	1 (50) 3	6 (75) 8	2 (67) 3	8 (89) 15	2 (67) 3	8 (89) 16	1 (50) 2	7 (78) 12	2 (67) 4	8 (100) 16	2 (67) 3		45 (76) 82	2 (33) 3
Injection site erythema				2 (22) 2	3 (100) 3	6 (67) 6		1 (11) 1	1 (33) 1	3 (38) 3	1 (33) 1		16 (27) 16	1 (17) 1
Injection site warmth		2 (25) 2	1 (33) 1	1 (11) 1	1 (33) 1	5 (56) 5		2 (22) 2		3 (38) 3	1 (33) 1	1 (33) 1	15 (25) 15	2 (33) 2
Injection site swelling		1 (13) 1	1 (33) 1		2 (67) 2	3 (33) 3	1 (50) 1	1 (11) 1		3 (38) 3			12 (20) 12	
Injection site pruritus		1 (13) 1		2 (22) 2	1 (33) 1	3 (33) 3				1 (13) 1			8 (14) 8	
Any solicited local reactions after TO vaccination (day 140 + 14 days)		2 (29) 2		4 (44) 8		2 (25) 4	1 (50) 4	1 (11) 1	1 (33) 1	3 (38) 5	2 (67) 3	1 (33) 1	14 (24) 25	3 (50) 4
Ocular discomfort		2 (29) 2		3 (33) 3		1 (13) 1	1 (50) 1			3 (38) 3	1 (33) 1		10 (17) 10	1 (17) 1
Ocular redness				2 (22) 2		1 (13) 1	1 (50) 1	1 (11) 1		2 (25) 2	1 (33) 1		7 (12) 7	1 (17) 1
Increased lacrimation				3 (33) 3		1 (13) 1	1 (50) 1				1 (33) 1		5 (9) 5	1 (17) 1
Swelling of eyelid						1 (13) 1	1 (50) 1		1 (33) 1			1 (33) 1	3 (5) 3	1 (17) 1
Any solicited systemic reactions after IM and ID/TO vaccination (day 112 + 14 days)		1 (13) 1		1 (11) 2	1 (33) 2	2 (22) 2							5 (9) 7	
Myalgia		1 (12·5) 1		1 (11) 1	1 (33) 1	2 (22) 2	1 (50) 1	1 (11) 1	1 (33) 1	1 (13) 1			9 (15) 9	
Chills				1 (11) 1	1 (33) 1			2 (22) 2	1 (33) 1	2 (2) 2			7 (12) 7	
Oral temperature > 38·3°C														
Rash														
Any adverse event(s) (AEs)	2 (100) 26	9 (100) 114	3 (100) 36	9 (100) 117	3 (100) 41	9 (100) 152	3 (100) 46	9 (100) 107	3 (100) 40	9 (100) 115	3 (100) 42	3 (100) 29	59 (100) 794	6 (100) 71
ADRs	2 (100) 17	9 (100) 78	3 (100) 24	9 (100) 92	3 (100) 38	9 (100) 112	3 (100) 37	9 (100) 82	3 (100) 35	9 (100) 93	3 (100) 23	3 (100) 14	59 (100) 608	6 (100) 37
AEs leading to drug withdrawal		1 (11) 4											1 (2) 4	
AEs leading to drug interruption	1 (50) 7			1 (11) 1		1 (11) 4		1 (11) 1		1 (11) 2			5 (9) 15	
AEs leading to withdrawal from trial														
Severity of AEs														
Mild (grade 1)	2 (100) 12	9 (100) 73	3 (100) 32	9 (100) 88	3 (100) 27	9 (100) 124	3 (100) 36	9 (100) 81	3 (100) 25	9 (100) 93	3 (100) 29	3 (100) 24	59 (100) 591	6 (100) 53
Moderate (grade 2)	2 (100) 12	8 (89) 39	2 (67) 4	6 (67) 28	2 (67) 14	8 (89) 28	2 (67) 10	8 (89) 26	3 (100) 15	8 (89) 20	3 (100) 13	3 (100) 5	49 (83) 196	6 (100) 18
Severe (grade 3)	1 (50) 2	2 (22) 2		1 (11) 1						2 (22) 2			6 (10) 7	

Relationship of AEs														
Not related	2 (100) 9	7 (78) 36	2 (67) 12	6 (67) 25	2 (67) 3	8 (89) 40	2 (67) 9	8 (89) 25	2 (67) 5	6 (67) 22	3 (100) 19	3 (100) 15	45 (76) 186	6 (100) 34
Related (Total)	2 (100) 17	9 (100) 78	3 (100) 24	9 (100) 92	3 (100) 38	9 (100) 112	3 (100) 37	9 (100) 82	3 (100) 35	9 (100) 93	3 (100) 23	3 (100) 14	59 (100) 608	6 (100) 37
Possibly related	1 (50) 3	5 (56) 12	2 (67) 4	4 (44) 7	2 (67) 5	6 (67) 15	1 (33) 8	6 (67) 10	1 (33) 7	5 (56) 7	2 (67) 11	3 (100) 4	33 (56) 78	5 (83) 15
Probably related	2 (100) 13	8 (89) 42	3 (100) 12	8 (89) 60	3 (100) 16	8 (89) 53	2 (67) 20	9 (100) 55	3 (100) 22	9 (100) 46	2 (67) 5	3 (100) 7	55 (93) 339	5 (83) 12
Certainly related	1 (50) 1	7 (78) 24	1 (33) 8	5 (56) 25	3 (100) 17	9 (100) 44	2 (67) 9	5 (56) 17	3 (100) 6	7 (78) 40	2 (67) 7	2 (67) 3	43 (73) 191	4 (67) 10
Outcome of AEs														
Recovered/resolved	2 (100) 25	9 (100) 110	3 (100) 35	9 (100) 113	3 (100) 41	9 (100) 149	3 (100) 42	9 (100·0) 100	3 (100·0) 39	9 (100) 110	3 (100) 42	3 (100·0) 27	59 (100) 764	6 (100) 69
Recovering/resolving		1 (11) 1		2 (22) 2		1 (11) 1	1 (33) 4	1 (11·1) 1		1 (11) 2			7 (12) 11	
Not recovered/not resolved	1 (50) 1	2 (22) 3	1 (33) 1	1 (11) 2		1 (11) 1		5 (55·6) 6	1 (33·3) 1	3 (33) 3		1 (33·3) 2	15 (25) 18	1 (17) 2
Unknown						1 (11) 1							1 (1·7) 1	

Table 2: CHLM-02 safety co-primary outcomes.

* Safety data from the day 0 of vaccination is presented grouped for arms A, B, C, D, E and F because they only received an IM vaccination only. In the later vaccination days, arms are divided into subgroups A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2 because subjects received different vaccinations by different administration routes in addition to the IM vaccination.

N: Number of participants experiencing the event at least once within 14 days after vaccination, %: Percentages were calculated based on the number of participants vaccinated at each visit E: Total number of reported events within 14 days after vaccination. Solicited systemic reactions are defined as AEs with the following preferred terms: pyrexia, chills, myalgia, and rash occurring within 14 days of vaccination. Only treatment emergent adverse events are presented and are defined as adverse events with onset at or after start of trial drug. IM: Intramuscular, ID: Intradermal, TO: Topical ocular, PCB: Placebo.

Cohort A1: 85 µg IM CTH522-CAF01 + ID Placebo, Cohort A2: 85 µg IM CTH522-CAF01 + TO Placebo, Cohort B: 85 µg IM CTH522-CAF01 + TO CTH522, Cohort C: 85 µg IM CTH522-CAF01 + ID CTH522, Cohort D1: 15 µg IM CTH522-CAF01 + TO Placebo,

Cohort D2: 15 µg IM CTH522-CAF01 + ID Placebo, Cohort E1: 85 µg IM CTH522-CAF09b + TO Placebo, Cohort E2: 85 µg IM CTH522-CAF09b + ID Placebo, Cohort F1: IM Placebo + TO Placebo, Cohort F2: IM Placebo + ID Placebo

Figure 1: CHLM-02 trial design

Screening →

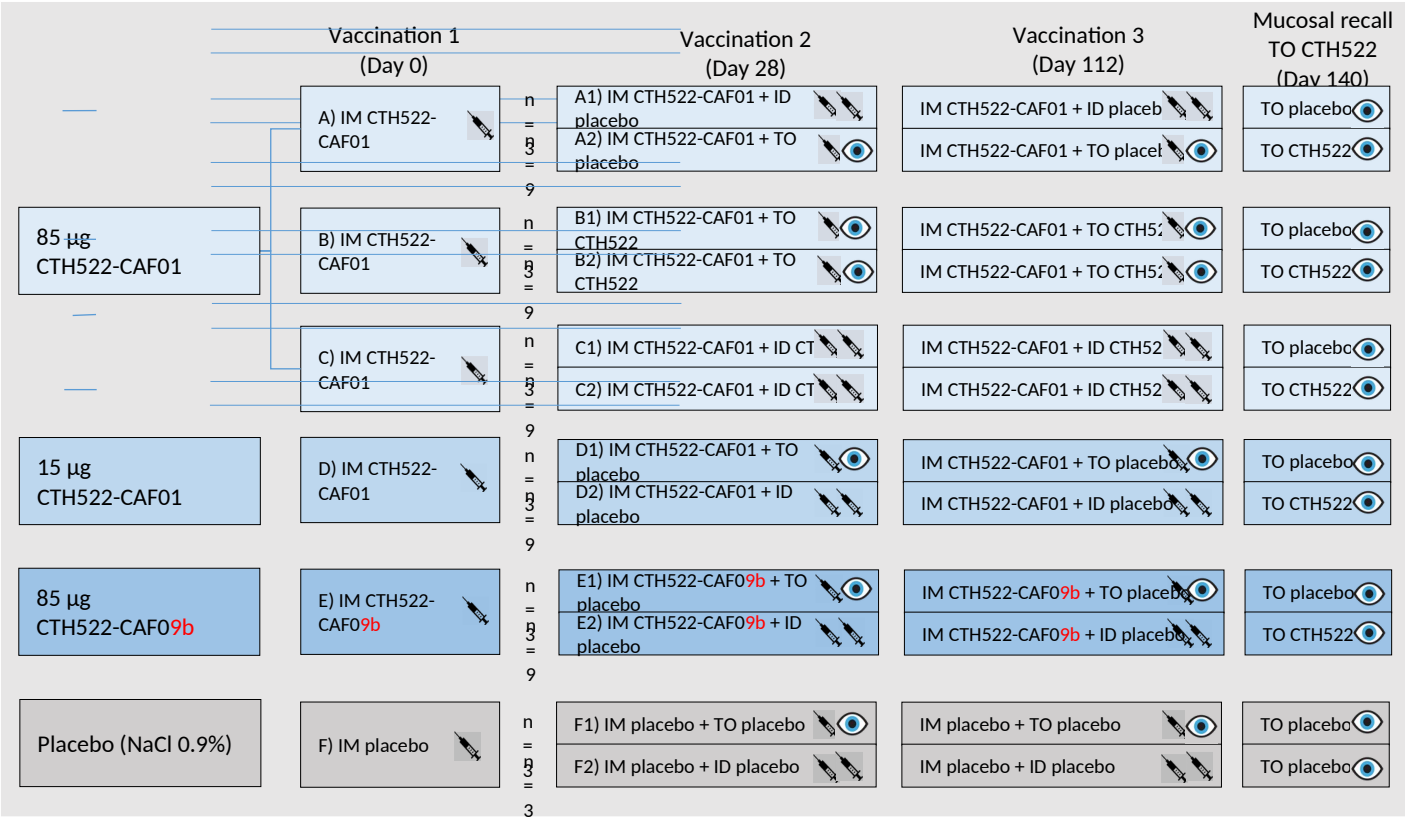


Figure 2: Median (IQR) anti-CTH522 serum IgG titre (intention to treat)

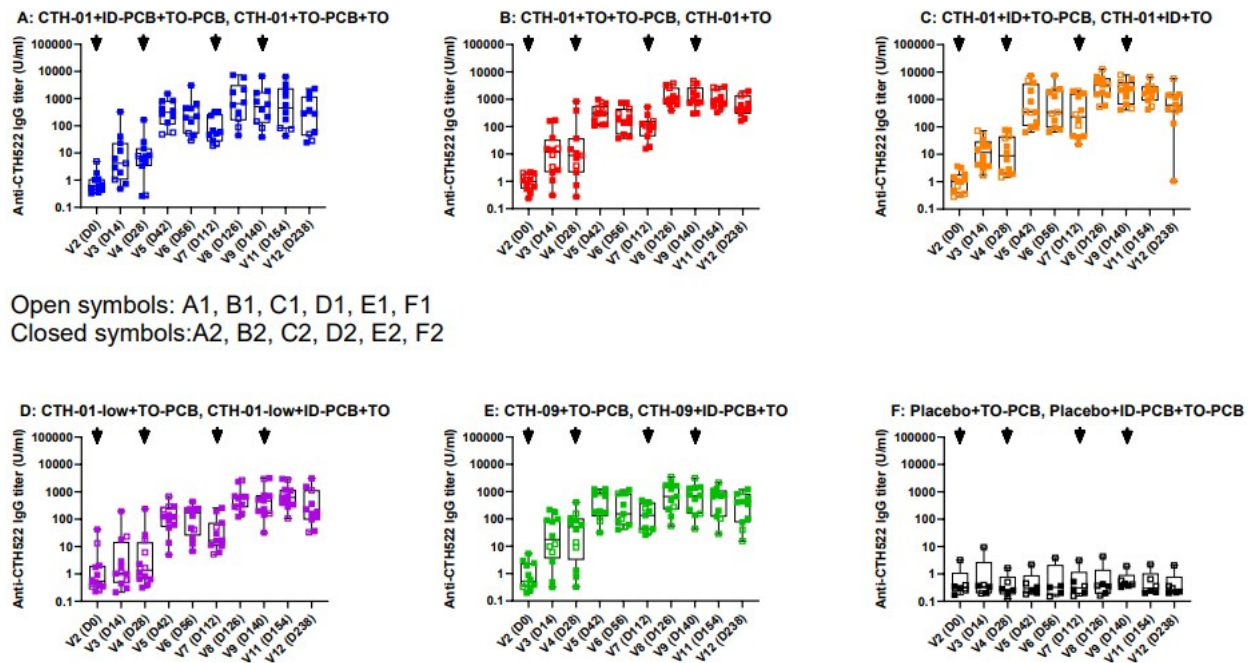


Figure 2: Median (IQR) anti-CTH522 IgG titre – full analysis set CHLM-02. Arms A (Blue), B (Red), C (Orange), D (Purple), E (Green) and F (Black) are shown. Open symbols represent Cohorts A1, B1, C1, D1, E1 and F1, where the TO placebo was administered. Closed symbols represent Cohorts A2, B2, C2, D2 and E2, where the active CTH522 was administered. Cohort F2 is also represented by closed symbols. The arrows indicate the timepoints at which the vaccines were administered.

Cohort A1: 85 µg IM CTH522-CAF01 + ID Placebo + TO Placebo, Cohort A2: 85 µg IM CTH522-CAF01 + TO Placebo + TO CTH522, Cohort B1: 85 µg IM CTH522-CAF01 + TO CTH522 + TO Placebo, Cohort B2: 85 µg IM CTH522-CAF01 + TO CTH522

Cohort C1: 85 µg IM CTH522-CAF01 + ID CTH522 + TO placebo, Cohort C2: 85 µg IM CTH522-CAF01 + ID CTH522 + TO CTH522, Cohort D1: 15 µg IM CTH522-CAF01 + TO Placebo, Cohort D2: 15 µg IM CTH522-CAF01 + ID Placebo + TO CTH522

Cohort E1: 85 µg IM CTH522-CAF09b + TO Placebo, Cohort E2: 85 µg IM CTH522-CAF09b + ID Placebo + TO CTH522, Cohort F1: IM Placebo + TO Placebo, Cohort F2: IM Placebo + ID Placebo + TO Placebo

Figure 3: CHLM-02 trial: total ocular IgA (intention to treat)

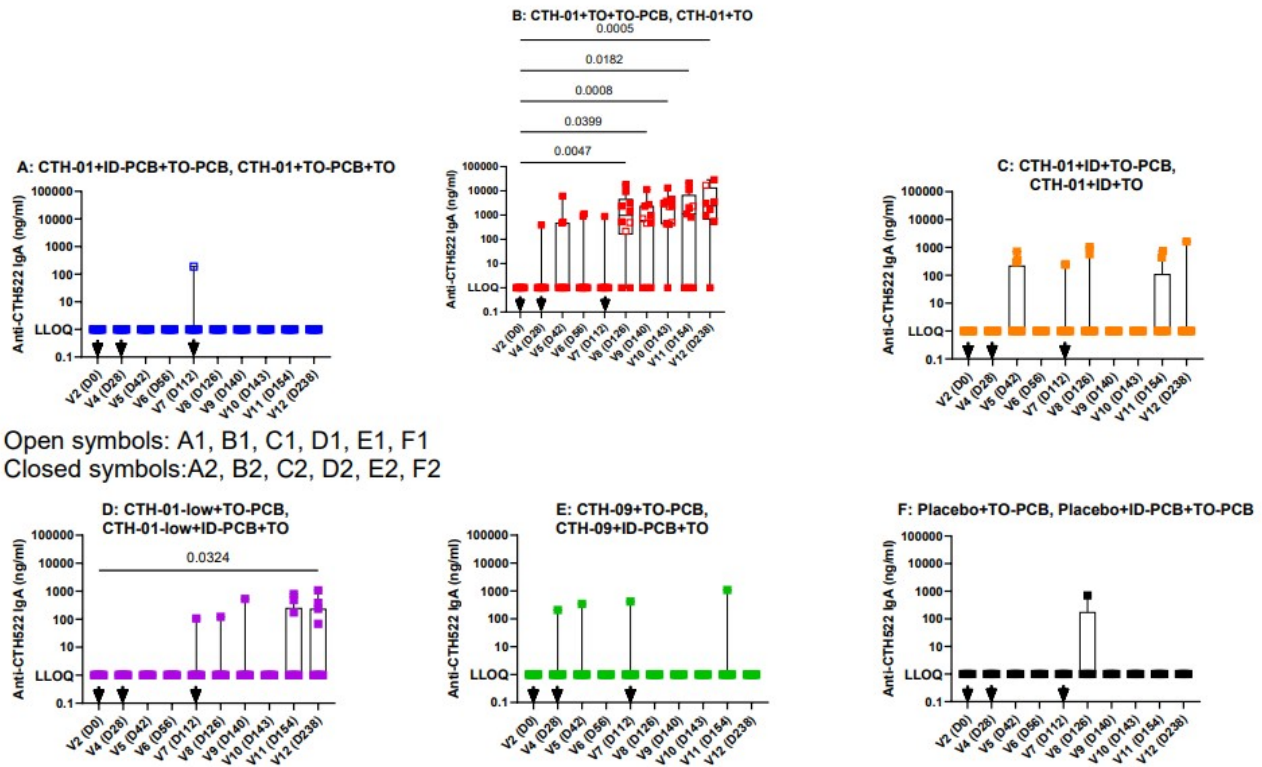


Figure 3: Concentration of CTH522-specific IgA antibodies in ocular samples from participants of the CHLM-02 vaccine trial.

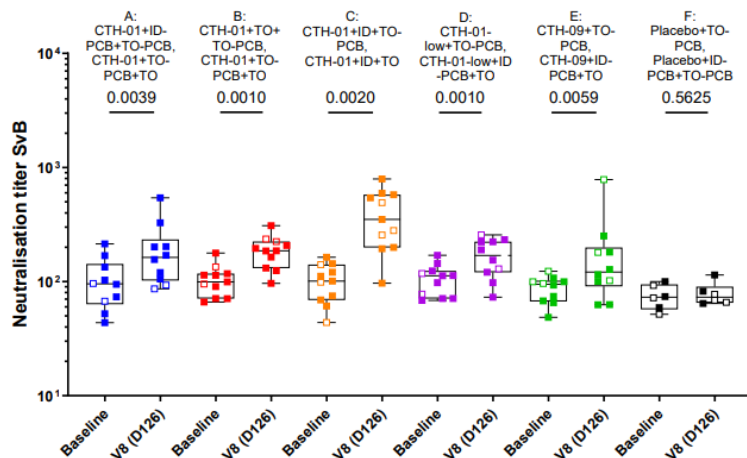
Cohorts A (Blue), B (Red), C (Orange), D (Purple), E (Green) and F (Black) are shown. Open symbols represent Cohorts A1, B1, C1, D1, E1 and F1, where the TO placebo was administered. Closed symbols represent Cohorts A2, B2, C2, D2 and E2, where the active CTH522 was administered. Cohort F2 is also represented by closed symbols. The arrows indicate the timepoints at which the vaccines were administered, with the clear arrow indicating TO administration. Median values with IQR are shown. Kruskal-Wallis Test with Dunn's multiple correction test performed. Statistical difference between baseline the remaining timepoints were compared.

Cohort A1: 85 µg IM CTH522-CAF01 + ID Placebo + TO Placebo,
Cohort A2: 85 µg IM CTH522-CAF01 + TO Placebo + TO CTH522,
Cohort B1: 85 µg IM CTH522-CAF01 + TO CTH522 + TO Placebo,
Cohort B2: 85 µg IM CTH522-CAF01 + TO CTH522
Cohort C1: 85 µg IM CTH522-CAF01 + ID CTH522 + TO placebo,
Cohort C2: 85 µg IM CTH522-CAF01 + ID CTH522 + TO CTH522,
Cohort D1: 15 µg IM CTH522-CAF01 + TO Placebo,
Cohort D2: 15 µg IM CTH522-CAF01 + ID Placebo + TO CTH522
Cohort E1: 85 µg IM CTH522-CAF09b + TO Placebo,
Cohort E2: 85 µg IM CTH522-CAF09b + ID Placebo + TO CTH522,
Cohort F1: IM Placebo + TO Placebo,

Cohort F2: IM Placebo + ID Placebo + TO Placebo

Figure 4: Serum CHLM-02 neutralising antibodies against serovars B and D median (IQR) titres (intention to treat)

A



B

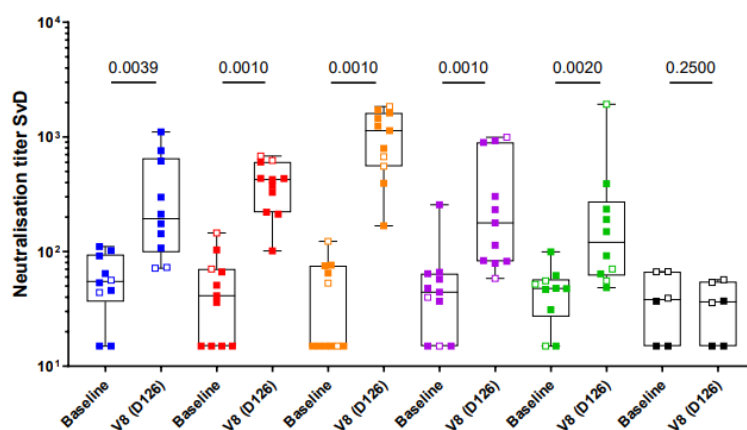


Figure 4: CHLM-02 neutralising antibodies against serovars B (A) and D (B) median (IQR) titres at Day 126. Arms A (Blue), B (Red), C (Orange), D (Purple), E (Green) and F (Black) are shown. Open symbols represent Cohorts A1, B1, C1, D1, E1 and F1, where the TO placebo was administered. Closed symbols represent Cohorts A2, B2, C2, D2 and E2, where the active CTH522 was administered. Cohort F2 is also represented by closed symbols.
 Cohort A1: 85 µg IM CTH522-CAF01 + ID Placebo + TO Placebo, Cohort A2: 85 µg IM CTH522-CAF01 + TO Placebo + TO CTH522, Cohort B1: 85 µg IM CTH522-CAF01 + TO CTH522 + TO Placebo, Cohort B2: 85 µg IM CTH522-CAF01 + TO CTH522
 Cohort C1: 85 µg IM CTH522-CAF01 + ID CTH522 + TO placebo, Cohort C2: 85 µg IM CTH522-CAF01 + ID CTH522 + TO CTH522, Cohort D1: 15 µg IM CTH522-CAF01 + TO Placebo, Cohort D2: 15 µg IM CTH522-CAF01 + ID Placebo + TO CTH522
 Cohort E1: 85 µg IM CTH522-CAF09b + TO Placebo, Cohort E2: 85 µg IM CTH522-CAF09b + ID Placebo + TO CTH522, Cohort F1: IM Placebo + TO Placebo, Cohort F2: IM Placebo + ID Placebo + TO Placebo

