# Safety and immunogenicity of chlamydia vaccine candidate CTH522 adjuvanted with CAF01 or AH: a first-in-human, randomised, double-blind, placebo-controlled, phase 1 study

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# Research in context panel

## Evidence before this study

We searched PubMed using the terms “chlamydia vaccine” and “clinical trial”, with no restrictions on publication dates (in essence January 1966 - January 2019) or language, and identified no reported studies. This is the first clinical trial of a genital chlamydia vaccine, and the first of a vaccine against *Chlamydia trachomatis* since the 1960s, when a number of studies assessed efficacy of live attenuated bacteria against ocular chlamydia infection (trachoma).

## Added value of this study

We found that intramuscular administration of CTH522 adjuvanted with either CAF01 or aluminium hydroxide, as well as intranasal administration of un-adjuvanted CTH522, was well-tolerated and immunogenic in healthy adult women. The vaccines induced high titres of serum antibodies and cell- mediated immune responses, measured as interferon (IFN)γ release. The antibodies were neutralising and were detectable in both the nasal cavity and genital tract. Of note, the CAF01 adjuvant induced superior antibody titres and cell-mediated immune responses, compared with aluminium hydroxide. Intranasal booster vaccination tended to increase IgA titres in both the nasal and genital tract secretions.

## Implications

The promising safety and immunogenicity profile of CTH522 adjuvanted with CAF01 encourages continued clinical development of this vaccine against genital chlamydia.

# Summary

## Background

Chlamydia is the most common bacterial sexually transmitted infection worldwide. National screening programmes and antibiotic treatment have failed to decrease incidence rates, and to date no vaccines against genital chlamydia have been tested in clinical trials. The aim of this study was to assess safety and immunogenicity in humans of a novel chlamydia vaccine based on a recombinant protein subunit (CTH522) in a prime-boost immunisation schedule.

## Methods

This phase I, first-in-human, double-blind, parallel, randomised and placebo-controlled trial performed in London, United Kingdom, included healthy women aged 19-45. Volunteers were randomly assigned to three groups in a 3:3:1 manner: CTH522 adjuvanted with CAF01 liposomes (CTH522:CAF01), CTH522 adjuvanted with aluminium hydroxide (CTH522:AH), or placebo. The participants received three intramuscular injections with adjuvant at months 0, 1 and 4, followed by two un-adjuvanted intranasal administrations at months 4·5 and 5. The primary outcome was safety and the secondary was humoral immunogenicity (anti-CTH522 IgG seroconversion). Clinicaltrials.gov: NCT02787109.

## Findings

Between July 2016 and February 2017, 35 volunteers were randomised and all (100%) were included in the study analyses (ITT). No related serious adverse reactions were reported, and the most frequent adverse events were mild local injection site reactions, which were reported in all (15/15) participants in the vaccine arms and in 60% of participants in the placebo group (3/5, p=0·0526 for both comparisons). Intranasal vaccination was not associated with a higher frequency of related local reactions (7/15 (47%) in the active treatment groups vs 3/5 (60%) in the placebo group (p=1·000)). Both CTH522:CAF01 and CTH522:AH induced anti-CTH522 IgG seroconversion in 15/15 (100%) participants after five immunisations, while no participants in the placebo group seroconverted. CTH522:CAF01 showed accelerated seroconversion, higher IgG titres, a superior mucosal antibody profile and a more consistent cell-mediated immune response profile.

## Interpretation

CTH522 adjuvanted with either CAF01 or AH was safe and well tolerated. Both vaccines were immunogenic, however CTH522:CAF01 demonstrated a superior immunogenicity profile holding promise for further clinical development.

## Funding

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# Introduction

The World Health Organisation estimates that more than one million new infections with the four curable sexually transmitted diseases (STDs) chlamydia, gonorrhoea, syphilis and trichomoniasis are acquired each day. With around 131 million annual incident infections, chlamydia remains the most common bacterial STD.1 The prevalence of chlamydia is age-dependent, with highest rates of laboratory-confirmed *Chlamydia trachomatis* infections in adolescents and young adults. However, as three in four infections remain asymptomatic, the incidence numbers are likely underestimated.1

Untreated or repeated infections are the main drivers of chlamydia-associated morbidity,2 which is estimated to cause the loss of 370,000 disability-adjusted life years annually.3 One in every six infected women develops ascending infection and pelvic inflammatory disease, which contributes to chronic pelvic pain and is a leading cause of tubal factor infertility and ectopic pregnancy, especially in the developing world.4 *C. trachomatis* infection is strongly associated with increased susceptibility to, and co-infection with, other STDs, in particular gonorrhoea and HIV.5 Infection during pregnancy poses a risk for adverse outcomes such as miscarriage, stillbirth, and preterm birth by either direct foetal infection, placental damage, or severe maternal illness.6 More than half of infants born to infected mothers become infected during birth, of whom one in six will develop pneumonia and around half conjunctivitis.7 In men, *C. trachomatis* mainly causes epididymitis, and in both sexes *C. trachomatis* infection can trigger reactive arthritis in a small minority of individuals.

Despite availability of both sensitive non-invasive tests and effective treatment, targeted screening and treatment programmes have, to a large degree, failed to curb the epidemic.8,9 Thus, an effective preventive vaccine would seem the best solution. Nevertheless, no vaccine against *C. trachomatis* has entered clinical trial since a series of trials against ocular chlamydia in the 1960s.

Studies of natural immunity suggest that infection can lead to partial and transient immunity to *C. trachomatis* characterised by both local humoral and cellular responses.10 Data from animal models point to a key role for IFNɣ-secreting Th1 cells and functional antibodies, preferably combined.11 However, it remains incompletely understood which mechanisms are necessary to target for a vaccine to confer protective immunity.

The vaccine antigen, CTH522, is a recombinant, engineered version of the *C. trachomatis* major outer membrane protein (MOMP), comprising heterologous immuno-repeats from four genital *C. trachomatis* serovars (D, E, F, and G).12 The pre-clinical research on this vaccine led to the selection of the cationic liposomal adjuvant CAF01 that has been designed for the induction of a strong cell-mediated immune response combined with antibody induction. The vaccine has been evaluated in mice, pigs and non-human primates where T cell responses and high titres of broadly neutralising antibodies were induced. Protection following genital *C. trachomatis* challenge was found in both mice12 and guinea pigs (unpublished).

Since the genital mucosa lacks immune inductive sites, other mucosal sites have been explored as alternative for the induction of local genital immunity, especially intranasal immunisation has been shown to induce mucosal immunity in both the respiratory and genital tract. Immunisation schedules with the adjuvant CAF01 have furthermore highlighted how systemic priming followed by mucosal boost is highly efficacious in inducing mucosal immunity and the induction of IgA.13-15

The aim of this trial was to assess the safety and immunogenicity of three intramuscular doses of CTH522 adjuvanted with CAF01 (CTH522:CAF01) or aluminium hydroxide (CTH522:AH), followed by two intranasal boosts with un-adjuvanted CTH522. The results will be used to guide further clinical development of the CTH522 vaccine.

# Methods

## Study design

This study was a phase 1, first-in-human, double-blind, parallel, randomised and placebo-controlled trial performed at the National Institute for Health Research (NIHR) Imperial Clinical Research Facility at Hammersmith Hospital in London, UK. The study protocol was approved by the London - Chelsea Research Ethics Committee, the Research and Development department at Imperial College Healthcare National Health Service (NHS) Trust, and the Medicines and Healthcare products Regulatory Agency (EudraCT number 2015-004330-10). The study was carried out in accordance with the International Conference on Harmonisation’s (ICH) Good Clinical Practices (GCP), and registered with ClinicalTrials.gov (NCT02787109).

## Participants

The study population comprised healthy women aged 18 to 45 years, who were not pregnant and agreed to use two approved forms of contraception or to complete abstinence during the trial period. The enrolled participants had body mass index <35 kg/m2, no history of pelvic inflammatory disease or other significant gynaecological diseases, had negative serological testing for HIV, hepatitis B, hepatitis C and syphilis, and negative urine PCR testing for *C. trachomatis* and gonorrhoea. Participants were excluded if they used an intrauterine device, were currently participating in another clinical trial, had clinically significant abnormality of haematological or biochemical parameters, received immunosuppressive treatment, or had received a vaccine within two weeks of the trial period. Participants were recruited through Imperial Clinical Research Facility’s healthy volunteers database, posters at NHS and university sites, and advertisements on social media. All participants gave written informed consent before enrolment.

## Randomisation and masking

The trial comprised three treatment groups, each with three intramuscular injections of adjuvanted vaccine (CTH522:CAF01 or CTH522:AH) or placebo (saline), followed by two intranasal administrations of un-adjuvanted CTH522 vaccine or placebo. The enrolled participants were randomly assigned to the treatment groups (in a 3:3:1 manner), via the eCRF system provided by the clinical research organization (Biostata, Allerød, Denmark) using a block size of 7. The randomisation module in the eCRF was set up by an unblinded person, not otherwise involved in the clinical trial. Unblinded trial staff members, who were not involved in any trial assessments, prepared and administered the vaccines. During trial drug administration, a blinded member of staff was also present to monitor any adverse events during or after vaccination. Participants, investigators, study nurses, laboratory personnel, and outcome assessors were all blinded to vaccine group allocation until database release.

## Procedures

The investigational recombinant protein vaccine CTH522 (batch number 528001), was produced under good manufacturing practice at Statens Serum Institut (Copenhagen, Denmark). The intramuscular dose of 85 µg CTH522 was administered to the deltoid region of the arm in a volume of 0·6 mL, containing either the liposomal adjuvant CAF01 (625 µg N,N´-dimethyl-N,N´-dioctadecylammonium (DDA) stabilised with 125 µg of the synthetic mycobacterial immunomodulator α,α´-trehalose-6,6´-dibehenate (TDB)) or 425 µg aluminium hydroxide (AH), both manufactured at Statens Serum Institut. The three intramuscular vaccinations were scheduled for day 0, month 1 (day 28) and month 4 (day 112). The intranasal dose of 2 x 30 µg CTH522 was administered to each nostril in a volume of 0·25 mL, using a VaxINator™ device (Teleflex Inc., PA, USA) at month 4·5 and 5 (appendix, figure 1S, page 9).

Safety was assessed after each vaccination as follows: daily completion of diary cards for 14 days, a telephone interview after 3 days, and a safety visit (vital signs and safety bloods) after 14 days. Collected information included: solicited local reactions to intramuscular vaccination (pain, erythema, tenderness, pruritus, warmth, stiffness and swelling), local reactions to intranasal vaccination (discharge, bleeding, congestion, discomfort, sneezing, cough), and systemic reactions to any vaccination (abnormally raised temperature (>38·3°C), chills, myalgia, malaise, fatigue, rash, headache, nausea and vomiting, and clinically significant abnormal values among full blood count, liver function test and renal profile results). Local and systemic adverse events were evaluated by a study clinician.

Samples for assessment of immunogenicity were collected at baseline and 1, 4, 4·5, 5, and 6 months after first immunisation for quantification of CTH522-specific IgG and IgA titres using ELISA, and at baseline and at 4·5 months for assessment of neutralising antibodies (see appendix for details). Peripheral blood mononuclear cells (PBMC) were collected at baseline and 4·5 months to determine cell-mediated immune (CMI) responses using IFNɣ ELISPOT (see appendix for details). Total mucosal IgG and IgA, and corresponding antibodies specific to CTH522 were quantified in nasal strips and vaginal fluid obtained using menstrual cup (Instead Softcup; EVOFEM Inc., CA, USA) samples collected at baseline and at 4·5, 5, and 6 months, using ELISA (see appendix for details). Additional samples for exploratory immunogenicity assessment were also collected (not reported here).

## Outcomes

The primary outcomes (safety) were solicited systemic reactions as well as solicited local reactions to intramuscular and intranasal vaccination recorded at any visit. The secondary outcome (humoral immunogenicity) was percentage of subjects achieving anti-CTH522 IgG seroconversion. Exploratory outcomes included evaluation of neutralising antibodies, mucosal antibody responses, antibody avidity and epitope use, and IFNɣ ELISpot, of which only the neutralising and mucosal antibody responses and IFNɣ ELISpot responses are included in this report.

## Statistical analysis

The sample size of 15 participants per vaccine arm and 5 subjects in the placebo arm which we considered adequate for a review of the safety profile of the described interventions. The study was not powered to detect differences between vaccine arms. All participants who had received at least one dose of vaccine were used in the analyses of the primary and secondary endpoints (endpoint analysis set). Safety results were expressed as the proportion of participants in each vaccine arm with adverse effects, in the three categories local injection site reactions, local nasal reactions, and systemic reactions, judged related or not related to study treatment, and compared with Fisher’s exact test. Seroconversion was defined as a 4-fold increase over baseline in specific serum IgG, and the proportions of seroconverted participants in each arm were compared with Fisher’s exact test. Confidence intervals for point estimates of effect size are presented as 95% unless otherwise stated. A post-hoc analysis of the amount of neutralising and mucosal antibodies as well as IFNɣ ELISpot results were presented as median and interquartile range (IQR), and compared with the Mann-Whitney *U* test. Correlation analysis was done using Spearman. CMI responder rates were defined as IFNɣ ELISpot responses above the mean baseline response of all volunteers + 3 standard deviations, and were compared between groups using Fisher’s exact test. The safety and seroconversion results were analysed using SAS version 9.4 following a predefined statistical analysis plan. The exploratory endpoints were assessed using R (version 3.5.1) with R studio (version 1.1.463). An independent data safety monitoring board was established to review and evaluate the trial data for participant safety and trial conduct.

## Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The sponsor of the study (Statens Serum Institut) participated in the study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

# Results

Between July 2016 and February 2017, 57 volunteers were screened and 35 randomised to receive CTH522:CAF01 (n=15), CTH522:AH (n=15), or placebo (n=5) (figure 1, table 1). Of the 35 participants, 32 (91%) received all five vaccinations described in the study protocol. Because of scheduling issues, two participants in the CTH522:CAF01 group withdrew from the study after three and five vaccinations, respectively. One participant in the CTH522:AH group withdrew after the second intramuscular vaccination because of low haemoglobin levels caused by a combination of a menorrhagia and study-related blood sampling (figure 1).

The primary endpoint was safety (see appendix page 4). No related serious adverse reactions occurred during the trial (table 2). The most frequently reported local reactions were injection site pain, tenderness and movement impairment, with 88-93% of events being reported as mild in each of the groups, lasting a median 2-4 days in all groups (range 1-11 days). All subjects recovered from all related adverse events. One unrelated serious adverse event occurred to a subject in the CTH522:CAF01 group (fracture of fibula following fall from a climbing wall).

All volunteers (15/15 (100%)) in the two active treatment arms experienced a local injection site reaction, which – although not significant – seemed to occur at a higher frequency compared to the placebo group (3/5 (60%), p=0·0526 for both comparisons, table 2). Intranasal vaccination was not associated with a higher frequency of related local reactions (7/15 (47%) in each of the active treatment groups vs 3/5 (60%) in the placebo group (p=1·000)), with the most frequent local reactions being sneezing, nasal congestion and rhinorrhoea. All but one event (moderate rhinorrhoea in the CTH522:CAF01 group) were of mild intensity.

The frequency of systemic adverse reactions did not differ between the three study arms, although there was a tendency towards a higher proportion of subjects in the two active treatment groups (10/15 (67%) in the CTH522:CAF01 and 13/15 (87%) in the CTH522:AH group), compared with 2/5 (40%) participants in the placebo group (p=0·3473 and P=0·0726, respectively). The most frequently reported systemic reactions were headache, fatigue, malaise, and myalgia.

Thirteen unsolicited treatment-emergent adverse reactions were reported (appendix, table 1S, page 10): five in the CTH522:CAF01 group, six in the CTH522:AH group and two in the placebo group. Among these were two cases of musculoskeletal stiffness (CTH522:AH group), two cases of oropharyngeal pain (one in CTH522:CAF01 and one in the placebo group), and two cases of nasopharyngitis (one in the CTH522:CAF01 and one in the CTH522:AH group).

For the secondary outcome of humoral immunogenicity, all 15 participants in the CTH522:CAF01 group (100%), 14 of 15 participants in the CTH522:AH group (93%) and none in the placebo group (0%) achieved the predefined >4-fold IgG seroconversion endpoint after the three intramuscular immunisations (appendix, table 2s, page 11). The nasal booster immunisations did not increase the systemic antibody levels. For the CTH522:CAF01 group, all seroconversions (15/15 (100%)) occurred after the second immunisation, and were sustained to the last time point (Appendix, table 2s, page 11).

The magnitude of IgG titres was assessed in a post-hoc analysis (figure 2A). Both vaccines generated strong responses already after the first immunization and responses increased with each intramuscular administration. Comparing median titres, CTH522:CAF01 induced a 5·6-fold higher median titre after the third intramuscular immunisation (p=0·0091), and remained >2·5-fold higher throughout the study.

The exploratory outcomes included assessment of neutralising antibody titres, mucosal antibodies, serum IgA and CMI responses. Both CTH522:CAF01 and CTH522:AH significantly increased the level of neutralising antibodies after the three intramuscular immunisations (p=0·000244 for both groups) (figure 2B). Although CTH522:CAF01 induced a higher median neutralisation titre than CTH522:AH (254·1 vs 107·4), there was no statistical difference between the two vaccines for this parameter. Anti-CTH522 serum IgA responses were significantly increased after intramuscular vaccination which continued after intranasal boost (appendix, figure S2, page 12), and highly correlated with serum IgG at month 4.5 and 6 (Spearman’s rho=0·63, p=0·0004068, and rho=0·78, p<0·0001, respectively (appendix, figure S3.A, page 13)).

Determination of mucosal antibody levels is difficult due to low antibody levels and sampling variability, therefore CTH522-specific IgG and IgA levels were normalised relative to total IgG and IgA levels in the sample, respectively. Antigen-specific vaginal IgG levels increased in the CTH522:CAF01 group (16·4-fold) after the intramuscular vaccinations and increased further following intranasal boost (p=0·027, figure 3A). Antigen-specific IgG increased in the nasal samples of both groups (CTH522:CAF01: 2·0-fold; CTH522:AH: 2·7-fold) after the intramuscular vaccinations, and increased further following intranasal boost in the CTH522:CAF01 group (p=0·040, figure 3C). No increase of mucosal IgG by intranasal boosting was seen in the AH-adjuvanted group (p=0·17). Mucosal IgA responses were only seen after intranasal boosting in the CTH522:CAF01 group (figure 3B and 3D). CTH522:AH did not promote IgA levels above background at any time point.

Mucosal IgG titres correlated strongly with serum levels (Spearman’s rho=0·89, p=<0·0001), whereas no such correlation was found between mucosal and circulating IgA levels (rho=0·18, p=0·43) suggesting some local production of IgA (appendix, figure S3B-C, page 13).

Vaccine-specific CMI responses were assessed using IFNɣ ELISpot at baseline and two weeks after three intramuscular vaccinations (figure 4). All participants had low baseline responses and in particular CTH522:CAF01 induced strong increases, with median values 252 spot-forming units (SFU)/1x106 cells (IQR 123-424 SFU/1x106 cells), which was higher than the CMI response induced by CTH522:AH (111 SFU/1x106 cells (IQR 70-269 SFU/1x106 cells)), although this difference did not reach statistical significance at the 95% level (p=0·05523 in a Wilcoxon rank sum test). All participants receiving the CAF01-adjuvanted CTH522 vaccine classified as responders (13/13), significantly more than for the AH-adjuvanted group, where only 57% (8/14) classified as responders (p=0·0101). There was a tendency, but no significant correlation between the IFNɣ ELISpot results and serum IgG titres (rho=0·38; p=0·051 at month 4·5, appendix figure S4, page 14).

# Discussion

We report the principal findings from a first-in-human clinical trial of the novel chlamydia vaccine CTH522. Results show that CTH522 adjuvanted with CAF01 liposomes or aluminium hydroxide (AH) administered with three intramuscular vaccinations and two intranasal boosts are both safe and immunogenic vaccines. No vaccine-related serious adverse events were reported and local reactions were mild and comparable with the safety profile of already licensed recombinant subunit vaccines.16 Intranasal boosting was not associated with a higher frequency of local reactions compared to placebo for any of the vaccines. The CAF01 adjuvant promoted higher antibody and CMI responses than AH. Furthermore, in contrast to AH, the CAF01-adjuvanted vaccine primed individuals for increased mucosal IgA after intranasal boost, albeit levels were low.

Given the impact of the chlamydia epidemic on women’s health, reproductive health, infant health through vertical transmission, and the increased susceptibility to other STDs, there is a global unmet medical need for a vaccine against genital chlamydia.17,18 Unfortunately, no surrogate endpoint for protection against chlamydia disease exists to guide development. However, based on studies of protection after natural infection, as well as various animal models, the prevailing paradigm is that an effective chlamydia vaccine ideally should generate a combined antibody and T cell response targeting genital epithelial cells.11

Some of the key features of this trial were the parallel assessment of two markedly different adjuvant systems, intranasal boost and the assessment of both systemic and mucosal immunogenicity. The trial was designed with an accelerated schedule of three intramuscular vaccinations given in a (0-1-4 month) prime-prime-boost schedule followed by two intranasal boosts with un-adjuvanted vaccine. In continuation of this trial, we are currently preparing a phase 2a trial, where this accelerated schedule will be changed into the classical prime-prime-boost schedule (0-1-6 month), developed for optimal B cell maturation and differentiation.19 This will also have the added benefit of aligning with the schedule for the human papilloma virus (HPV) vaccine, which targets the same age group.

CTH522:CAF01 was consistently more immunogenic compared to CTH522:AH, inducing a 5·6-fold higher IgG titre after the third intramuscular immunisation, as well as stronger mucosal and cell mediated immune responses. The IgG titres induced by CTH522:CAF01 are therefore comparable to other licenced recombinant protein vaccines including the adjuvanted hepatitis B vaccine,16 although the absence of a correlate of protection renders such comparisons speculative. The ability of CAF01 to facilitate antibody responses has been assessed in other human trials with varying outcome. A CAF01-adjuvanted recombinant tuberculosis vaccine candidate H1 induced no antibodies, but this vaccine contained considerably less antigen than in the present study.20 A malaria vaccine GMZ, on the other hand, generated a strong antibody response on par with AH.21 AH is considered the gold standard for antibody inducing vaccines,22 and it was thus unexpected to see CAF01 surpass AH on all the serological parameters.

When administered intramuscularly in mice, CAF01 induces an immune response characterised by Th1 and Th17 cells, which is an ideal profile for induction of mucosal B cells and a secretory (s-)IgA response.15 Vaccine studies in mice14 and mini-pigs13,15 have demonstrated a cross-mucosal immunological link between nasopharyngeal and genital mucosal immunity, and this trial was - in part - designed to confirm this link in humans. Although mucosal responses were low, in particular in the nasal samples, we were able to detect significant increases in vaccine-specific responses with the CAF01-adjuvanted vaccine. Interestingly, IgA responses were unique to the CAF01 adjuvant and seemed to be dependent on the intranasal boost, as would have been predicted based on the extensive animal model data available for this this vaccine.

Significant amounts of specific IgG were found in the vaginal fluid in both vaccine groups correlating well with serum levels. IgG antibodies in the female genital tract are primarily thought to be derived from serum,23 and our findings support that circulating IgG antibodies reach the genital tract in high titres. The results are in line with observations from the human papilloma virus (HPV) vaccines, where measurable vaginal IgG antibodies are detectable at various time points post last vaccination.24,25 Efficacy of the HPV vaccines is well established and the major mechanism of protection is thought to be transudation of serum antibodies into cervical secretions. The relative role of IgA and IgG for protection against *C. trachomatis* is not clear, but the promising results in the present study prompt further exploration of the relative role of these isotypes in later trials.

Neutralising vaginal antibodies are the first line of defence against *C. trachomatis* infection and are thought to be a key to the protective efficacy of CTH522. Adoptive transfer studies of antibodies in mice have shown that neutralising antibodies can block infection and also act in synergy with cellular immune responses.12,26 We have established an *in vitro* inhibition assay which correlates with the ability of antibodies to protect against the first phase of infection in animal models.12,26 Significant levels of neutralising antibodies were found in both CTH522:CAF01 and CTH522:AH-vaccinated individuals in the clinical trial. CTH522:CAF01 induced a higher median neutralisation titre than CTH522:AH, and for both groups there was a strong correlation with the serum titres against CTH522 (Spearman's rank correlation coefficient = 0·74). If this correlation is reproduced in confirmatory clinical trials, it would be tempting to use the plasma titres as a simple surrogate for the functional assay. However, neutralisation will most likely not capture the full picture of the protective mechanism behind CTH522-induced functional antibodies, especially the ability of antibodies to recruit the cellular immune response via Fc receptors.27 Ongoing studies will characterise the antibody function in opsonisation, complement activation and antibody-dependent cellular cytotoxicity. This will aid in identifying potential correlates of protection further on in clinical development.

Comprehensive preclinical evidence support the role of cellular immunity and in particular IFNɣ-secreting Th1 cells in the elimination of intracellular bacteria.28 CTH522 contains numerous T cell epitopes from MOMP,29 and dissection of the CTH522:CAF01 protective response in animal models suggests an important synergistic role of CD4+ T cells and neutralising antibody responses.12 In the present clinical trial, we observed a robust cellular response measured by the number of vaccine-specific IFNɣ-secreting T cells. These results are in line with previously published IFNɣ ELISpot results with CAF01 used in a vaccine against tuberculosis, and, worth noting in this regard, in that clinical trial CAF01 had the ability to maintain immunological memory with stable CMI responses for more than 150 weeks.20

One consideration as this vaccine moves into more advanced clinical evaluation, is vaccine coverage against clinically relevant strains. CTH522 incorporates a key neutralising epitope expressed in serotypes D-G, which are the most prevalent serotypes in clinical circulation, representing up to 90% of infections.30 Importantly, the CTH522 vaccine molecule additionally contains large segments of MOMP shared among all genital tract isolates, and these segments of the molecule are known to contain both shared B and T cell epitopes.29 If the CTH522:CAF01 vaccine demonstrates proof of concept in a future clinical efficacy trial, the vaccine may therefore potentially provide some level of protection against the remaining 10% clinically relevant serovars.

This study had several limitations. As with other phase 1 studies, the small sample size limited the assessment of rare adverse events and prevented well-powered immunological investigations.

The accelerated schedule probably resulted in suboptimal antibody maturation, and a wider spacing between the second and third intramuscular immunisations could possibly have generated superior neutralising antibody responses.19 The chosen sampling strategy did not allow for clarification of whether the intranasal boosts were the exclusive driver of the mucosal IgA response. However, it is reassuring to find significant induction of antigen-specific responses at the mucosal sites which should inspire further pursuit, however, the further clinical development will address if a complex regimen with mucosal boost is required.

Our trial did not include participants with a history of *C. trachomatis* infection, however, given the high prevalence of unacknowledged infections, a potential impact of already established infection or adaptive immunity on vaccine safety and immunogenicity will be a priority in future clinical assessments of this vaccine. Finally, as there is no established correlate of protection against Chlamydia, it remains unknown if the immune response generated by the CTH522 based vaccines correlate with protective immunity. This remains a point for future study.

In conclusion, we demonstrate that CTH522:CAF01 and CTH522:AH are both safe and immunogenic. The superior immunogenicity profile of CTH522:CAF01 warrants further clinical development and the preparation of a phase 2 dose optimisation study is currently ongoing.

## Contributors

SA conducted the clinical trial, with assistance from TC. RJS, PA and FRF designed the study and analysis plans with input from MR, IK, MPK, KSK and KM. HBJ and MR wrote the first draft of the paper, with input from FF, and analysed the immunogenicity data. HC processed and stored all samples, and performed the ELISpots and mucosal ELISAs, together with LM and SD. PB managed the clinical trial. RBD compiled the safety and immunogenicity data. MPK and KSK qualified and performed the serum ELISA, KSK designed the ELIspot analysis and analysed the results. SK performed the neutralisation assay, with input from AO and HBJ. DL, KM, MR, IK, PA, RS, RJS, KSK, MPK, HBJ and FF discussed and interpreted the overall dataset. All authors have read and approved the final version.

Conflicts of interests

SA, HBJ, PB, RBD, TC, HMC, MPK, KSK, DL, LRM, SD, SK, KM, IK, RJS declare no conflicts of interests. PA, AWO and FF are co-inventors on a patent application on Vaccines against Chlamydia sp. [WO2014146663A1]. All rights have been assigned to Statens Serum Institut, a Danish not-for-profit institute under the Ministry of health.

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Figure Legends

Figure 1: Trial profile, AH=aluminium hydroxide.

Figure 2: Serology measurements

Change in (A) anti-CTH522 serum IgG ELISA units and (B) neutralising antibody titres over time. The box illustrates the IQR, with a horizontal line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon signed rank test p values are shown. For serum IgG, the titres remained significantly higher than baseline for the duration of the study for both active vaccines, but for clarity only selected comparisons are indicated. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisation. AH=aluminium hydroxide.

Figure 3: CTH522-specific mucosal antibody responses

Change in vaginal IgG (A), vaginal IgA (B), nasal IgG (C), and nasal IgA (D) from baseline to 2 weeks after the third intramuscular immunisation (month 4·5), 2 weeks after the first intranasal immunisation (month 5·0), and 4 weeks after the second intranasal vaccination (month 6·0). Values are shown as CTH522-specific IgG or IgA as a proportion of corresponding total IgG or IgA. Boxes show IQR, with a black line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon signed rank test p values are shown for nasal antibodies, and because of missing values at some time points Wilcoxon rank sum test p values are shown for vaginal antibodies. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisations. AH=aluminium hydroxide.

Figure 4: Cell-mediated immune responses

Interferon-γ spot-forming units (SFU) for each participant at baseline and at month 4·5 were assessed by use of enzyme-linked immunopot (CTH522:CAF01 [nine of 13 participants], CTH522:AH [12 of 14], and placebo [four of five]). 0·2 × 106 peripheral blood mononuclear cells were stimulated in triplicates with either medium alone or 5 μg/mL CTH522 for 24 h. Presented values are spot counts after protein stimulation, which have been subtracted from the spot counts after medium stimulation. Boxes show IQR, with a black line at the median value; whiskers show 1·5 × IQR, and dots represent outliers. Wilcoxon rank sum test p values are shown. The vaccine schedule is shown above the x-axis, with grey triangles indicating intramuscular immunisations, and white triangles indicating intranasal immunisations. AH=aluminium hydroxide.

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![A screenshot of a social media post

Description automatically generated]()

Figure 1



Figure 2



Figure 3



Figure 4

|  |  |  |  |
| --- | --- | --- | --- |
|  | CTH522:CAF01 (n=15) | CTH522:Alum (n=15)1 | Placebo (n=5) |
| Age (years) | 24 (19-42) | 26 (19-43) | 23 (22-45) |
| Ethnicity or race [n (%)] |  |  |  |
| White | 9 (60%) | 10 (67%) | 4 (80%) |
| Asian | 3 (20%) | 3 (20%) | - |
| Black | 2 (13%) | 2 (13%) | 1 (20%) |
| Other | 1 (7%) | - | - |
| BMI (kg/m2) | 23·0 (18·6-34·9) | 23·1 (18·7-27·9) | 22·1 (20·0-31·3) |
| Baseline anti-CTH522 IgG (U/mL) | 0·98 (0·35-25·45) | 1·24 (0·31-34·95) | 2·58 (0·63-8·46) |

*Table 1*: Baseline characteristics of the per protocol population

BMI=body-mass index. Data are given as median (range), unless otherwise indicated.

Table 1

|  |  |  |  |
| --- | --- | --- | --- |
|  | Treatment groups | | |
|  | **CTH522:CAF01 (n=15)** | **CTH522:Alum (n=15)** | **Placebo (n=5)** |
| Any related adverse event\* | 15 (100%) 202 | 15 (100%) 182 | 4 (80%) 23 |
| Solicited injection site reactions (LLT) | 15 (100%) 126 | 15 (100%) 106 | 3 (60%) 14 |
| Pain | 14 (93%) 33 | 9 (60%) 19 | 1 (20%) 2 |
| Tenderness | 14 (93%) 34 | 14 (93 %) 30 | 2 (40%) 2 |
| Movement impairment | 14 (93%) 31 | 13 (87%) 26 | 2 (40%) 5 |
| Redness | 6 (40%) 9 | 7 (47%) 9 | 1 (20%) 1 |
| Warmth | 5 (33%) 11 | 5 (33%) 9 | 2 (40%) 3 |
| Swelling | 4 (27%) 5 | 5 (33%) 5 | 1 (20%) 1 |
| Itching | 2 (13%) 3 | 6 (40%) 7 | 0 |
| Muscle reaction | 0 | 1 (7%) 1 | 0 |
| Solicited local reactions after intranasal vaccination (PT) | 7 (47%) 30 | 7 (47%) 18 | 3 (60%) 3 |
| Sneezing | 2 (13%) 5 | 5 (33%) 8 | 1 (20%) 1 |
| Nasal congestion | 4 (27%) 6 | 3 (20%) 5 | 0 |
| Rhinorrhoea | 6 (40%) 9 | 1 (7%) 1 | 0 |
| Epistaxis | 2 (13%) 4 | 1 (7%) 1 | 0 |
| Nasal discomfort | 1 (7%) 2 | 1 (7%) 1 | 1 (20%) 1 |
| Throat irritation or oropharyngeal pain | 2 (13%) 2 | 0 | 1 (20%) 1 |
| Cough | 1 (7%) 1 | 1 (7%) 1 | 0 |
| Ear discomfort or ear pain | 1 (7%) 1 | 1 (7%) 1 | 0 |
| Solicited systemic reactions | 10 (67%) 41 | 13 (87%) 52 | 2 (40%) 4 |
| Headache | 9 (60%) 19 | 9 (60%) 16 | 2 (40%) 3 |
| Sinus headache | 0 | 1 (7%) 1 | 0 |
| Fatigue | 5 (33%) 8 | 8 (53%) 9 | 1 (20%) 1 |
| Malaise | 6 (40%) 9 | 4 (27%) 6 | 0 |
| Myalgia | 2 (13%) 3 | 4 (27%) 5 | 0 |
| Nausea | 0 | 4 (27%) 8 | 0 |
| Rash | 1 (7%) 1 | 3 (20%) 4 | 0 |
| Chills | 1 (7%) 1 | 2 (13%) 3 | 0 |

*Table 2*: Related solicited adverse events in the 14 days after each vaccination.

All data are shown as [n (%) events]. LLT = Lowest Level Term. PT=Preferred Term. There were no significant differences in any of the comparisons; p-values are reported in the main text. \*See table 1S for details on the 13 unsolicited related adverse events.