# Part B: Information about the release application to be included on the public register

## B1 The name and address of the applicant

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## B2 A general description of the genetically modified organisms in relation to which the application is being made

Description of the GMO

The GMO is an isogenic mutant of a wild-type *Salmonella* Paratyphi (*S*. Paratyphi) A 9150 strain. The purpose of the genetic modification is to construct a modified *S*. Paratyphi A 9150 strain carrying the deletion of the *guaBA* operon, and *clpX* gene, to generate a growth-deficient, attenuated *S*. Paratyphi A strain (CVD 1902). The GMO (CVD 1902) will be used to investigate its value as a live attenuated oral vaccine to protect against enteric fever in a controlled human infection model.

CVD 1902 *Salmonella enterica* serovar Paratyphi A live oral vaccine was constructed from wild type parent strain *S.* Paratyphi A 9150. Deletions were carried out using a modified Lambda Red-mediated site-directed mutagenesis procedure. Two genetic sequences were deleted: the *guaBA* chromosomal operon (which encodes two enzymes employed in the distal *de novo* guanine nucleotide biosynthesis pathway) and *clpX* gene (which encodes a chaperone ATPase that functions with the serine protease encoded by *clpP* to form a complex that participates in a variety of metabolic processes). One of the phenotypic consequences of a deletion mutation in *clpX* is the hyperexpression of flagella which may also enhance the immunogenicity of the GMO strain and therefore contribute to its suitability as a live attenuated vaccine. Information derived from the study will be used to inform vaccine design and development potentially influencing public health intervention strategies.

Description of the application

In this clinical study we propose to investigate the efficacy of the GMO CVD 1902 as a live attenuated vaccine to prevent paratyphoid infection within the *S.* Paratyphi A human challenge model. The human infection model of S. Paratyphi A infection using wild-type *S*. Paratyphi A (NVGH308 strain) has been established at the Oxford Vaccine Group (University of Oxford, UK). The

Oxford Vaccine Group (University of Oxford, UK) has been undertaking controlled human challenge studies using *S.* Typhi and *S.* Paratyphi A since 2010.

The GMO to be released is intended for use in healthy adults aged 18-55 years. The total sample size will be 66-76 participants. Participants will be randomized to receive two doses of the GMO (at a dose of not less than 2 x $10^{10}$ CFU per dose) or two doses of placebo (bicarbonate solution) administered 14 days apart. Twenty-eight days after receiving their second dose participants will be 'challenged' with wild-type *S*. Paratyphi A (NVGH308 strain). Following challenge participants will be treated with antibiotics. Antibiotic therapy is initiated either at the onset of paratyphoid fever or 14 days after challenge, whichever occurs sooner.

The primary objective of the study is to compare the proportion of participants developing clinical or microbiologically proven paratyphoid infection following wild-type challenge in the CVD 1902 vaccinated group compared with the placebo group. Secondary objectives include the safety and tolerability of the vaccine strain and immune response to the vaccine.

## B3 The location at which the genetically modified organisms are proposed to be released

The address of the proposed site of release is: Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE. The national (OS) grid reference for the proposed site release is SP543060.

As a consequence of shedding through faecal material the GMO may be released into the sewage system in England and primarily within the Oxfordshire area. The sewage system is designed to contain and clear bacteria including *Salmonell*a.

## B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

The purpose of the study is to utilise the human volunteer challenge model to investigate the efficacy of a live oral attenuated *S.* Paratyphi A vaccine (CVD 1902). Information derived from this study will be used to inform vaccine design and development, potentially influencing public health intervention strategies.

The primary objective of the study is to compare the proportion of participants developing clinical or microbiologically proven paratyphoid infection following wild-type challenge in the CVD 1902 vaccinated group compared with the placebo group. Secondary objectives include the safety and tolerability of the vaccine strain and immune response to the vaccine.

## B5 The intended dates of the release.

The exact timing of the release will be dependent on all study approvals being in place. We anticipate that clinical study will commence on the 1st of January 2022 and recruitment may take up to 48 months (until 1st of January 2026). A total of 66-76 participants will be enrolled to the study from within the Oxfordshire area. The GMO will be given orally to study participants who are likely to shed the organism in faeces/stool. This shedding constitutes the release of the GMO. The duration of the release (involving both oral challenge and the shedding phase of the study) is expected to be no longer than 48 months.

The duration of the release which includes both dosing (vaccination) and shedding phase of the study will be no longer than 48 months (with completion in January 2026). Monitoring will take place for the duration of the clinical study. Due to the evolving global situation with respect to coronavirus the duration time for this study is lengthy as it is unclear exactly at what point it will be feasible to commence this study. We anticipate the trial will run for at least 48 months but this may be longer, for example if the start is significantly delayed, if there are difficulties with recruitment or if follow on studies involving the release of this GMO are required, hence release may occur until September 2028.

## B6 The environmental risk assessment.

The GMO is an isogenic mutant of the wild-type (parent) *S*. Paratyphi A 9150 strain and has been manufactured to GMP standard for use as an oral vaccine within a controlled human infection model to be conducted at the Oxford Vaccine Group (University of Oxford, UK). The GMO will be administered orally to healthy volunteers recruited to the study who are likely to shed the organism in faeces/stool. This will constitute the release of the organism resulting in release into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person-to-person transmission. The GMO is expected to be effectively contained and inactivated by usual sewage system processes.

Wild-type *S*. Paratyphi A is a human restricted pathogen with no known animal reservoir and the GMO will not have a selective or survival advantage. The potential for genetic exchange with any other organism in the environment is extremely low given that the GMO does not contain any plasmids or antibiotic resistance markers. All participants will receive a course of antibiotic treatment following challenge with the wild-type *S*. Paratyphi A challenge strain. The GMO is not expected to persist in study participants following the completion of 7 days of antibiotic treatment. Stool clearance will be confirmed following 3 negative stool samples provided at least 48 hours apart following completion of a 7 day course of antibiotics.

For the purpose of the release, the GMO will be given to a group of 33-38 participants at a single site in Oxford. Additional participants may receive the GMO to account for participant withdrawals before the completion of antibiotic treatment period of the study. The study participants will be healthy adult volunteers aged 18-55 years.

The GMO has previously been administered to human subjects in a phase I clinical trial conducted at the University of Maryland Center for Vaccine Development in the USA. To date no toxic or allergenic effects were reported following administration of the GMO to adult human volunteers in the human challenge model. The risk of both the GMO and wild-type strain to other humans is considered to be low and the risk to the environment is considered to be effectively zero.

## B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

Methods and plans for monitoring the GMO

The health and safety of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate.

Stool cultures will be taken at Day-42 (first vaccination), D-35, D-28, (second vaccination), D-21 and at Day-0 (challenge), daily throughout the 14 day post-vaccination period and at visits after paratyphoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Following antibiotic treatment participants will be required to supply clearance stool samples until proven not to be shedding *S.* Paratyphi A. Stool samples will be collected at least one week after completion of a 7 day course of antibiotics, until three successive stool samples are negative for *S*. Paratyphi A. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust. Additionally, quantitative stool cultures or PCR may be performed to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include phage typing or genetic sequencing.

Frequency of the monitoring

Monitoring will take place for the duration of the clinical study. We anticipate that clinical study will commence on the 1st of January 2022 (pending all necessary approvals) and recruitment may take up to 48 months (until 1st of January 2026).

All study participants will have follow-up visits up to 1-year post challenge. Following vaccination with the GMO strain participants will be monitored remotely via e-diary and in clinic visits. Following challenge with wild-type

strain participants are reviewed at daily clinic visits for 14 days post-challenge. Continuous participant safety monitoring will occur throughout the vaccination and challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary. The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling will be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90, 180 and 365. All study participants will agree to have 24-hour contact with study staff during the vaccination and challenge period and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

Blood samples will be monitored daily for *S*. Paratyphi A using a combination of microbiological and molecular biology techniques. Organisms which will be identified as *S*. Paratyphi A via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard microbiological methods.

Participants will be screened for shedding of *S*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-28, (second vaccination), D-21 and Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Paratyphi A. To detect chronic carriage of *S*. Paratyphi A, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If stool samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of chronic carriage or transmission to secondary contacts of the wild type *S*. Paratyphi A NVGH308 strain after treatment has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with wild-type *S.* Paratyphi A and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participant's GP will also be notified of trial entry, challenge with wild-type *S*. Paratyphi A and stool shedding clearance. In addition any breaches in enteric precautions that result in another individual coming into contact with the excreta of a participant will be reported to Public Health England.

Stringent precautions are in place to avoid the spread of the GMO from the study participant to others. Such spread has not been noted in previous

studies conducted by the Oxford Vaccine Group using the wild type *S*. Paratyphi NVGH308 strain. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing). In view of the low infectivity of *S.* Paratyphi (without administration in sodium bicarbonate buffer) and the high standard of hygiene and sanitation in the UK, secondary transmission of the challenge strain to household or other close contacts is highly unlikely. It is acknowledged, however, that transmission within households can occur if the individual excreting *S.* Paratyphi A fails to practice effective hand washing after defecation and is subsequently involved in uncooked food preparation. The participant will provide letters from the study team to close contacts including household contacts. Contacts will be offered the opportunity to be screened for *S*. Paratyphi A infection, which will involve obtaining two stool samples 48-hours apart. If either stool culture of a household contact is positive, he/she will be referred to a Consultant in Infectious Diseases for appropriate antibiotic management and Public Health England will be informed.

Methods and plans for responding to an emergency

Participants who experience vomiting for any reason within 60 minutes of the vaccination/placebo or will be withdrawn from the trial and assessed as to whether treatment with antibiotics is required. This will be treated as an emergency spill of the GMO and standard operating procedures will be followed by the research team. Suitable personal protective equipment and disinfectants will be used to inactive the GMO. All waste will be autoclaved prior to disposal from the site according to local GMO standard operating procedures.

Participants will be instructed to notify the study team of any serious adverse events/reactions following administration of the GMO. All participants agree to have 24-hour contact with study staff during the vaccination period and until four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion. A physician from the clinical team will be on-call 24 hours. In addition, participants agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S.* Paratyphi A strain (GMO and wild type NVGH308 strain), study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (ciprofloxacin, azithromycin, or other macrolide antibiotics, co-trimoxazole, ceftriaxone) will be excluded from the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with side effects. Should an antibiotic cause allergy or

intolerance this will be managed by a study doctor and a different antibiotic will be used for subsequent management. The participant's GP will be notified in writing of the antibiotics received. Participants will receive telephone calls or text messages to remind them to take their antibiotic dose.

There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to the John Warin Ward (or other suitable inpatient facility, John Radcliffe Hospital, Oxford) in cases of severe paratyphoid fever and/or other circumstances.