BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072938
Article Type:	Protocol
Date Submitted by the Author:	02-Mar-2023
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Keywords:	Clinical Trial, IMMUNOLOGY, Public health < INFECTIOUS DISEASES

Salmonella Vaccine Study in Oxford (SALVO) Trial: Protocol for an Observer-Participant Blind Randomised Placebo-Controlled Trial of the iNTS-GMMA Vaccine within a European Cohort

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Keywords:
Clinical Trial
Non-typhoidal Salmonella
Vaccine
Invasive salmonellosis

Abstract

Introduction

Invasive non-typhoidal Salmonellosis (iNTS) is mainly caused by *Salmonella enterica* serovars Typhimurium and Enteritidis and is estimated to result in 77,000 deaths per year, disproportionately affecting children under 5 years of age in sub-Saharan Africa. Invasive non-typhoidal *Salmonellae* serovars are increasingly acquiring resistance to first line antibiotics, thus an effective vaccine would be a valuable tool in reducing morbidity and mortality from infection. While NTS livestock vaccines are in wide use, no licensed vaccines exist for use in humans. Here, a first-in-human study of a novel vaccine (iNTS-GMMA) containing *S.* Typhimurium and *S.* Enteritidis Generalized Modules for Membrane Antigens (GMMA) outer membrane vesicles is presented.

Method and Analysis

The *Salmonella* Vaccine Study in Oxford (SALVO) is a randomised placebo-controlled participant-observer blind phase 1 study of the iNTS-GMMA vaccine. Healthy adult volunteers will be randomised to receive 3 intramuscular injections of the iNTS-GMMA vaccine, containing equal quantities of *S.* Typhimurium and *S.* Enteritidis GMMA particles adsorbed on Alhydrogel, or an Alhydrogel placebo at 0-, 2- and 6-months. Participants will be sequentially enrolled into 3 groups: Group 1, 1:1 randomisation to low dose iNTS-GMMA vaccine or placebo; Group 2, 1:1 randomisation to full dose iNTS-GMMA vaccine or placebo;

Group 3, 2:1 randomisation to full dose or lower dose (dependant on DSMC reviews of Group 1 and 2) iNTS-GMMA vaccine or placebo

The primary objective is safety and tolerability of the vaccine. The secondary objective is immunogenicity as measured by O-antigen based enzyme linked immunosorbent assay. Further exploratory objectives will characterise the expanded human immune profile.

Ethics and Dissemination

Ethical approval for this study has been obtained from the South Central - Oxford A Research Ethics Committee (Ethics REF:22/SC/0059). Appropriate documentation and regulatory approvals have been acquired. Results will be disseminated via peer reviewed articles and conferences.

Registration Details

EudraCT Number: 2020-000510-14

Article Summary

Strength and Limitations

- SALVO is a first in human study of a novel vaccine against invasive non-typhoidal Salmonellosis (iNTS), a neglected disease largely affecting low- and middle-income countries.
- This study provides the opportunity to investigate the adaptive immune response to bacterial outer membrane antigens, supporting future vaccine development efforts against this disease.
- The volunteers recruited to this trial may not be immunologically representative of the ultimate target population in endemic areas. In addition the vaccination schedule in this study is based on the expected schedule in infants.
- In the absence of a known correlate of protection against iNTS disease, it will not be possible to determine vaccine efficacy in this trial.

Introduction

Non-typhoidal *Salmonellae* (NTS), such as *Salmonella enterica* serovars Enteritidis and Typhimurium, most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens (1). However, some NTS bacterial strains can also cause an invasive syndrome with bacteraemia, high fevers, and metastatic infection which if untreated can lead to septicaemia and death. Invasive non- typhoidal *Salmonella* (iNTS) infections are more common in children, the elderly and in the immunosuppressed, including HIV-infected individuals (2,3).

The Global Burden of Disease study estimates 535,000 annual cases of iNTS globally, associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella* (1,4). The highest burden of iNTS disease globally occurs in sub-Saharan Africa, with a pooled annual incidence of 52/100,000 (1,4). This is likely an underestimate given the limited availability of diagnostics in the region coupled with non-specific disease presentation. The age at which infection occurs shows a bimodal distribution in most African studies with 68.3% occurring in children under 5 years and a second peak in the 30-40 years age group, believed to be associated with HIV, malaria, and malnutrition (3,5–7).

Of the invasive pathogens responsible for iNTS in sub-Saharan Africa, *S*. Typhimurium is implicated in approximately two-thirds of all cases, with the ST313 serotype accounting for most isolates (8). In contrast to other non-invasive strains, African ST313 isolates often exhibit genomic degradation and pseudogene formation like that seen in typhoidal *Salmonellae*, which contribute to human host restriction and an invasive phenotype (9–12). Furthermore, iNTS strains such as ST313 have been associated with multi-drug resistance, leading to *Salmonellae* being classified as WHO high priority antibiotic resistant pathogens (13–15).

High mortality, logistical difficulties in diagnosing infection in the developing world and increasing antimicrobial resistance strongly advocate for the development of an effective vaccine.

There are no currently licensed vaccines for iNTS although multiple candidates are in early phase development, including O-antigen (OAg) conjugates, oral attenuated vaccines, and multiple antigen display protein-polysaccharide conjugate vaccines. A trivalent iNTS-typhoid vaccine is currently in phase 1 (16).

The investigational product in this study is the iNTS-GMMA vaccine. This novel vaccine developed by GSK Vaccines for Global Health (GVGH) consists of outer membrane vesicles or Generalized Modules for Membrane Antigens (GMMA) of the two most common serotypes associated with invasive disease, *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) (17). Generalized Modules for Membrane Antigens (GMMA) particles contain several immunodominant antigens including the OAg component of bacterial lipopolysaccharide and outer membrane proteins. iNTS-GMMA are immunogenic in animal models, eliciting antibodies directed against OAg and demonstrating serum bactericidal

activity. Immunised animals also appear to have lower systemic bacterial loads on subsequent challenge (18).

This is the first trial to investigate the iNTS-GMMA vaccine in humans. Demonstration of safety and immunogenicity in this study will lead to progression to subsequent studies in a sub-Saharan country of high endemicity.

Study aims and objectives

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine and study the immune response to vaccination. Primary, secondary, and exploratory objectives are detailed in Table 1.

Objective		Outcome measure
Primary	To determine the safety and tolerability of the iNTS-GMMA vaccine	Clinical review and participant recording of solicited, unsolicited adverse events, serious adverse events, withdrawals, and laboratory parameters (haematology/biochemistry)
Secondary	To investigate immunogenicity of the iNTS-GMMA vaccine	Measurement of serovar specific O-antigen by ELISA before and after vaccination
Exploratory	To further characterise the immune response to vaccination	Exploratory immunological analyses including functional antibody assays and antigen-specific memory B cell responses and T cell responses before and after vaccination

Table 1 Primary, secondary, and exploratory objectives and outcomes of the SALVO study.

Methods

Trial interventions: IMP and placebo

The iNTS- GMMA vaccine consists of outer membrane vesicles or GMMA from the two most common serovars causing invasive disease, *Salmonella* Enteritidis and S*almonella* Typhimurium adsorbed onto Alhydrogel (0.35 mg AL^{3+} /0.5 mL dose) and suspended in isotonic phosphate buffered saline. The parent bacteria have been genetically modified to increase production of outer membrane vesicles (Δ*tolR*) and reduce the toxicity of lipid A component within the lipopolysaccharide (Δ*msbB* and Δ*pagP*). The GMMA particles are filtered and purified to form the active component of the vaccine (19–21). Two dose levels will be used for this study: a full dose of 20 µg STmGMMA + 20 µg SEnGMMA (total 40 µg O- antigen); and a lower dose consisting of 5.3 µg STmGMMA + 5.3 µg SEnGMMA (total 10.6 µg OAg). The placebo matches the vaccine matrix and consists of Alhydrogel without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

Study design and setting

This is a first-in-human randomised placebo-controlled participant-observer blind trial of the iNTS-GMMA vaccine in healthy adults aged 18-55 years in the United Kingdom. A total of 30-42 participants will be randomised to receive 3 intramuscular doses of active vaccine or placebo at 0, 2 and 6 months (Figure 1). For Further details please see SALVO Protocol in Supplementary Material 1.

Figure 1 SALVO vaccine and visit schedule

Participants will be sequentially enrolled into three groups (Figure 2) with a dose escalation between Group 1 (lower dose iNTS-GMMA vaccine, 10.6 µg total OAg content) and Group 2 (full dose iNTS-GMMA vaccine, 40 µg total OAg content). These first two groups will each consist of six participants who will be randomised 1:1 to the active vaccine or placebo. An additional six participants may be recruited to each of these groups if further safety information is required. Group 3 consists of 18 participants randomised 2:1 to receive the iNTS-GMMA vaccine or placebo. The decision to proceed to low or full dose vaccine in Group 3 will be based on safety reviews of Groups 1 and 2. There will be external safety monitoring reviews by the Data Safety Monitoring Committee (DSMC) between the two dose escalation groups and at a further two time-points in group three.

Figure 1 SALVO study design

Randomisation

Randomisation of participants will be carried out by unblinded study staff who are independent from the blinded team and do not perform any post vaccination procedures (such as ongoing eligibility or safety review). A web-based randomisation system will be used.

Blinding

This study will be conducted observer- and participant-blind from the time of randomisation until participant unblinding which will occur once the last participant has completed their final visit. Observer and participant blinding is required to minimise the risk of bias on the reporting of adverse events following the administration of vaccine.

Study visits

Vaccine or placebo will be administered at 0, 2 and 6 months. Participants will be directly observed for a minimum of 60 minutes following vaccination and then asked to complete an e- diary of their symptoms daily for 7 days following each vaccination. An in-person post-vaccination review will occur 7 and 28 days following each vaccine when participants will be reviewed for any possible adverse events. There are a total of 12 scheduled study visits and participants will be followed up for 1 year following first vaccination.

Recruitment and eligibility

Potential participants may be contacted by media advertisements, direct mail out or social media using an approved invitation letter or other approved advertising material to invite them to participate in the study. Participants will be reimbursed for their time, travel, and inconvenience. Healthy adults between the ages 18-55 years inclusive will be eligible for enrolment. Individuals will be initially screened for eligibility by telephone followed by face-to-face visits at the trial centre. Screening visits will involve obtaining informed consent (See SALVO Informed Consent Form in Supplementary Material 2), application of inclusion and exclusion criteria (summarised in Table 2) and clinical eligibility assessments including vital signs, physical examination, baseline blood tests, and urinalysis (SALVO Protocol in Supplementary Material 1).

Table 2 Summary of SALVO inclusion and exclusion criteria

Inclusion criteria	Willing and able to give informed consent for participation in the study
	Aged between 18-55 years inclusive
	In good health as determined by: - Medical history - Physical examination - Laboratory assessment - Clinical judgement of the investigators
	Willing to use highly effective contraception from one month prior to receiving the first vaccine and for the duration of the study (Females)
	Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
	Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study
	Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS)
Exclusion criteria	History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators.
	Have any known or suspected impairment or alteration of immune function.
	Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing
	Prior history of receipt of an oral typhoid vaccine (e.g.: Ty21a) within the last three years or a paratyphoid vaccine (as part of a clinical trial)
	Prior history of participation in a Typhoid or Paratyphoid controlled human infection study
	Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
	Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (except for COVID-19 vaccines)
	History of allergy or anaphylaxis to a previous vaccine or vaccine components
	Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
	Participation in another research study involving an investigational product or that which may compromise the integrity of the study
	Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
	Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study
	Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m2

	Any other significant disease or disorder which, in the opinion of the Investigator, may: - Put the participants at risk because of participation in the study - Influence the result of the study - Impair the participant's ability to participate in the study

Sample Size and Statistical Analysis

The sample size in this study is 30-42 participants to account for additional participants to be recruited on DSMC advice. As the initial phase 1 trial primary objective is safety and tolerability this sample size has been chosen to evaluate early data on adverse events associated with increasing dose-level, with a larger subsequent phase 1 trial in a highly endemic country planned dependant on the trial safety data. The statistics for the primary endpoint are descriptive, with no testing of statistical significance. The confidence intervals will be set at 95%.

Ethics and Dissemination

As a first-in-human vaccine trial the iNTS-GMMA vaccine has undergone appropriate pre-clinical toxicology studies indicating a well-tolerated vaccine. Participants will be actively monitored for their safety during the trial by review of an electronic diary, visits, clinical observations, and safety blood tests and will have access to a 24-hour medical contact number. Appropriate risk and benefits of the study will be communicated to the participants, and informed consent will be taken prior to any study related procedures. Local and national guidelines on confidentially and data protection will be adhered to.

The DSMC consisting of an experienced group of clinicians and a statistician will be appointed to provide real-time independent oversight of safety and trial conduct. The DSMC will review safety data collated from participant and clinician recorded entries including solicited and unsolicited adverse events, laboratory results and vital signs. Progression of enrolment from Group 1 to Group 2 to Group 3 will only occur after DSMC review of the relevant safety data. Further DSMC reviews will occur regularly throughout the trial. A development safety update report (DSUR) for the IMP will be prepared annually, on the anniversary of the MHRA approval for the trial.

Undertaking non-COVID-19 research during a dynamic COVID-19 pandemic represents a major logistical challenge. The safety of participants remains paramount and good infection prevention and control practices will be followed throughout the trial. The SALVO study team will monitor cases of COVID-19 within the participant cohort and the local population and will follow current national guidelines on COVID-19 with options including switching to phone appointments, halting, or extending the trial.

Once the trial has been completed including analysis of data, results will be published in a peer-reviewed journal and presented at conferences. The results of this study will directly impact the appropriateness of subsequent trials with a larger sample size to begin in a sub-Saharan country of high iNTS endemicity.

This study has been approved by South Central - Oxford A Research Ethics Committee on 28th April 2022.

Patient and public involvement statement

The protocol, study information booklet and recruitment materials were reviewed by a local patient consultation group who provided feedback and comments on the initial documents. Their comments led to changes in the participant-facing documents, ensuring they are easy and clear for participants to understand (Please see SALVO Participant Information Sheet in Supplementary Material 3).

Discussion

This will be the first phase 1 study investigating the iNTS-GMMA vaccine. The data generated by this trial will guide future vaccine development using this GMMA technology and may contribute to the licensure of the first vaccine against invasive non-typhoidal *Salmonella* species.

iNTS disproportionately causes severe disease in children under the age of 5 years in sub-Saharan Africa and an effective vaccine will be of greatest benefit in this vulnerable population. As the UK is a country with a low burden of non-typhoid *Salmonella* disease (22,23), healthy volunteers recruited to the SALVO study will be unlikely to have pre-existing immunity to *S*. Typhimurium or *S*. Enteritidis. However, assessment of the immunogenicity of the iNTS-GMMA vaccine in an immunologically naïve cohort in SALVO will inform the decision to progress to a second larger phase 1 trial in a sub-Saharan African population where iNTS is endemic. Future studies will recruit African infants, in whom any future licensed vaccine is likely to be deployed.

As GMMAs originate from the bacterial outer membrane they contain both the immunodominant OAg as well as multiple membrane proteins in their native conformation (19). Alongside measurement of OAg binding antibody after vaccination, this study will use functional antibody assays (including serum bactericidal activity), to interrogate the outer membrane protein specific responses. This may reveal conserved proteins present across multiple *Salmonella* serovars capable of eliciting pan-protective immune responses.

SALVO will be the first clinical trial of the iNTS-GMMA vaccine. However, GVGH is also developing another enteric vaccine using the GMMA technology. The *Shigella sonnei*-GMMA vaccine (1790GAHB) has undergone five clinical trials including three phase 1 studies in Europe (24,25), one phase 2a in sub-Saharan Africa (26) and a single phase 2b controlled human infection model in the US (27) and is well-tolerated and immunogenic. Currently the only other iNTS-based vaccines to enter clinical trials are two trivalent vaccines covering *S. enterica* serovars Typhimurium, Enteritidis and Typhi (28,29) with results to be published. A live attenuated oral *S.* Typhimurium was trialled in participants in 2009 but has not progressed further in the intervening years (30). Further iNTS-based vaccines are progressing through the pre-clinical phase including flagellin and OmpD based vaccines (31).

Ultimately, the geographical and demographic overlap of infection caused by typhoidal and iNTS serovars in Africa suggests that a pan-*Salmonella* or multivalent vaccine would have significant global public health value. iNTS vaccines are likely to be incorporated into national immunisation schedules in Africa alongside a typhoid conjugate component as a multi-valent vaccine. The SALVO study will thus provide critical information on the iNTS GMMA-based technology to guide the next generation of *Salmonella* vaccines.

The study protocol was prepared in accordance with the SPIRIT 2013 Checklist (32).

Authors' contributions

BH and MR designed and authored the protocol. BH and NK wrote the manuscript. NO, CF, HR, RW, PF, UN, RC, AJP contributed to the protocol design and/or study set up.

Conflicts of Interests

The iNTS-GMMA vaccine has been provided by the GSK Vaccines Institute for Global Health (GVGH). GVGH has reviewed the protocol developed by the Oxford Vaccine Group, University of Oxford and provided funding for clinical trial monitoring.

PF, UN and RC were employees of the GSK Vaccines Institute for Global Health at the time in which the study was conducted. UN owns shares in GSK. GSK Vaccines Institute for Global Health Srl is an affiliate of GlaxoSmithKline Biologicals SA. This does not alter the authors' adherence to all Journal policies on data and material sharing.

Funding

This research was funded in whole or in part by EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission.

Data Statement

The Protocol Version 3.1 January 2023 has been published in supplementary material 1.

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Word Count 2636

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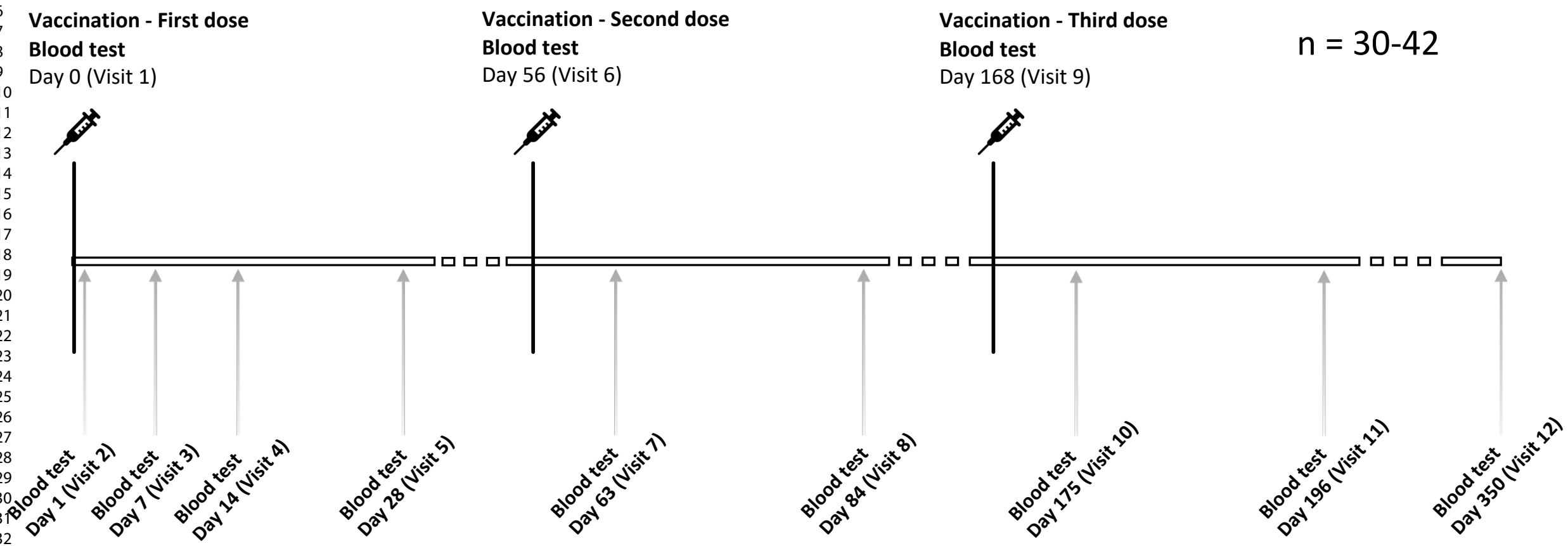
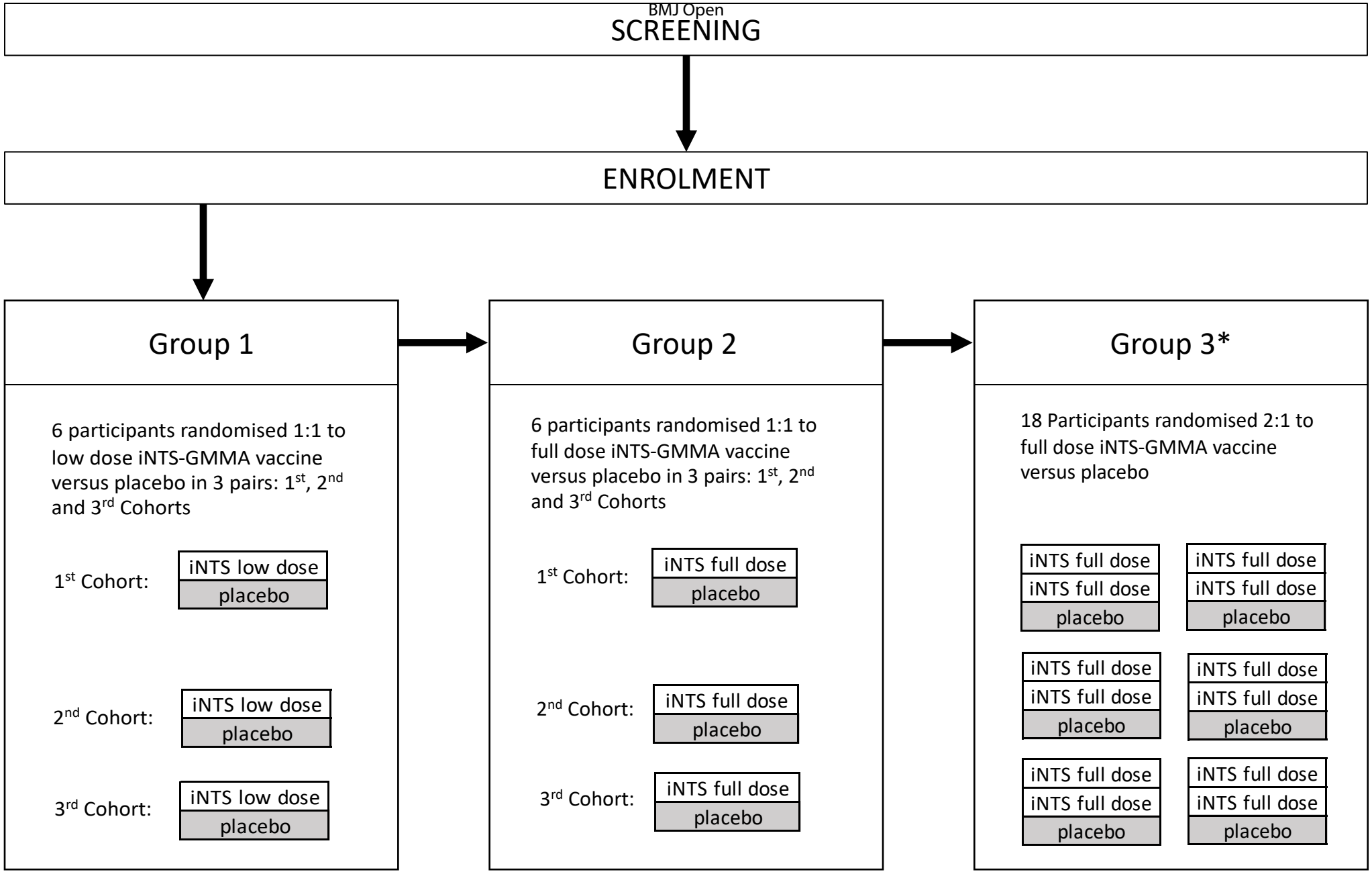

Vaccination - First dose
Blood test
Day 0 (Visit 1)
Vaccination - Second dose
Blood test
Day 56 (Visit 6)
Vaccination - Third dose
Blood test
Day 168 (Visit 9)
n = 30-42
Blood test Day 1 (Visit 2)
Blood test Day 7 (Visit 3)
Blood test Day 14 (Visit 4)
Blood test Day 28 (Visit 5)
Blood test Day 63 (Visit 7)
Blood test Day 84 (Visit 8)
Blood test Day 175 (Visit 10)
Blood test Day 196 (Visit 11)
Blood test Day 350 (Visit 12)



**Decision to proceed to low or full dose will be based on safety reviews of Groups 1 and 2.*

Trial Title: A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella

Internal Reference Number / Short title: OVG2020/01 Salmonella **V**accine Study in **O**xford (SALVO)
Ethics Ref: 22/SC/0059
IRAS Project ID: 1005098
EudraCT Number: 2020-000510-14
Date and Version No: 13 January 2023 / Version 3.1

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Sponsor: University of Oxford
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The GSK Vaccines Institute for Global Health (Monitoring Costs Only)

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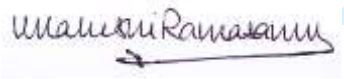

Statistician Signature:

We declare no financial conflict of interest in this study.

Confidentiality Statement
This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

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Funder(s)	EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439
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LAY SUMMARY

Nontyphoidal Salmonellae are types of bacteria that can cause gut infections resulting in diarrhoea, both in the UK and globally. However, under some circumstances, these bacteria can cause a more severe illness where infection spreads beyond the gut into the blood stream, a condition termed invasive non-typhoidal Salmonellosis (iNTS). iNTS disease is an under-recognised cause of disease and death in Sub Saharan Africa. In these regions, it primarily occurs in young children, particularly those with malaria and malnutrition. High death rates, difficulties in diagnosing this infection in the developing world, increasing resistance of the bacteria to common antibiotics, and spread via contaminated food and water make development of an effective and affordable vaccine against iNTS an essential control measure.

A new and innovative vaccine (iNTS-GMMA), has been developed which is based on the formation of bacterial outer surface particles. This vaccine facilitates exposure of components of the bacteria to the human immune system without the risk of causing infection. Developed by GSK Biologicals and GSK Vaccines Institute for Global health (GVGH), the aim of this vaccine is to confer immune protection to the most common African strains of the bacteria causing iNTS disease.

This study is a first-in-human clinical trial involving 30-42 healthy adult participants who will be randomly allocated to receive either iNTS-GMMA or a placebo. The main objective of this trial is to evaluate the safety of the iNTS-GMMA vaccine in healthy adults in the UK. The secondary objective is to investigate the human immune response to iNTS-GMMA vaccine.

3 SYNOPSIS

Trial Title	**A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella**
Internal ref. no. (or short title)	**OVG2020/01 Sal**monella **V**accine Study in **O**xford (SALVO)
Trial registration	ISRCTN51750695
Sponsor	University of Oxford Research Governance, Ethics and Assurance Joint Research Office Boundary Brook House Churchill Drive Headington Oxford OX3 7GB United Kingdom
Funder	EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS The GSK Vaccines Institute for Global Health (Monitoring Costs Only)
Clinical Phase	Phase I, First in Humans
Trial Design	Single centre, participant-observer blind, randomised placebo-controlled safety and immunogenicity interventional study
Trial Participants	Healthy adults, aged 18-55 years inclusive
Sample Size	• 30-42 participants. • Up to 12 participants randomised 1:1 to receive lower dose at 10.6µg (3.8 dilution of full dose) OAg of iNTS GMMA vaccine or a placebo. • Up to 12 participants randomised 1:1 to receive the full dose at 40µg OAg of iNTS GMMA vaccine or a placebo • Eighteen participants randomised 2:1 to receive either the lower dose or full dose dependant on DSMC review versus placebo.
Planned Trial Period	• Total trial period including data analysis is 36 months. • The intended duration of a participant on the trial is 12 months. • The total duration from first participants first visit to last participants last visit is maximum 18 months.
Primary Objective	To determine the safety and tolerability between two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each

	adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years inclusive when given three doses of vaccine at 0, 2- and 6-month intervals.
Secondary Objectives	To investigate the immunogenicity at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Exploratory Objectives	To further investigate the immunogenicity using exploratory immunological analyses at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Primary endpoint	The recording and assessment of local and systemic adverse events following administration of each vaccine dose: 1. Tenderness and pain at the injection site 2. Induration 3. Redness 4. Swelling 5. Headache 6. Malaise 7. Myalgia 8. Nausea and/or vomiting 9. Diarrhoea 10. Abdominal Pain 11. Anorexia 12. Arthralgia 13. Fatigue 14. Fever 15. Blood parameters (haematology / biochemistry) 16. Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR
Secondary endpoints	Immunological assays to study the immune responses to vaccines, including:

	1. Antibody concentration against serovar specific O antigen determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
Exploratory endpoints	Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to: 1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose. 2. Serum bactericidal antibody (SBA) titre against vaccine homologous strains before and after each dose 3. Serum bactericidal antibody (SBA) titre against a panel of other strains before and after each dose 4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose 5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose 6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose 7. Investigate the innate and adaptive response to the iNTS-GMMA vaccine by utilising next generation sequencing of the transcriptome to evaluate the differential gene expression profile and DNA storage for investigation of the genetic associations with the immune response 8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose 9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays 10. Faecal antibody concentration against O antigen determined by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection. 11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.
Investigational Medicinal Products	1. iNTS GMMA vaccine 2. Comparator (Placebo)
Form of vaccine	Glass vials containing 0.7ml sterile suspension containing either STmGMMA (80 μg/mL in OAg) or SEnGMMA (80 μg/mL in OAg) formulated with Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5

Form of Comparator (Placebo)	Glass vials containing 0.7ml sterile suspension containing Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH6.5
Dose	Lower Dose: 5.3 µg (OAg) STmGMMA/Alhydrogel + 5.3 µg (OAg) SEnGMMA/Alhydrogel (3.8x dilution of full dose generated by combining 0.5 mL of the two vaccine components into an empty vial and transferring 0.25 mL of the two components into 0.7 mL of the placebo vial, 0.5mL of combined vaccine to be administered) Full Dose: 20 µg (OAg) STmGMMA/Alhydrogel + 20 µg (OAg) SEnGMMA/Alhydrogel (generated by combining equal volumes of the two vaccine components in an empty vial, 0.5 mL of combined vaccine to be administered) Comparator (Placebo): 0.5mL to be administered
Route	Intramuscular
Vaccine Schedule	3 doses given at 0, 2 and 6 months

ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CCVTM	Centre for Vaccinology & Tropical Medicine
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DMSC	Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
ELISA	Enzyme Linked Immunosorbent Assay
GCP	Good Clinical Practice
GMMA	Outer membrane exosome from genetically modified Gram negative bacteria used as an antigen delivery system; GMMA is a pun on the Italian word for jewel or bud.
GP	General Practitioner
GVGH	GSK Vaccines Institute for Global health
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
iNTS	Invasive Non-typhoidal Salmonella
IRB	Independent Review Board
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
OVG	Oxford Vaccine Group
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PBMC	Peripheral Blood Mononuclear Cell
RGEA	Research Governance, Ethics and Assurance

R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SEn	*Salmonella* Enteritidis
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
STm	*Salmonella* Typhimurium
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TOPS	The Over volunteering Prevention System (http://www.tops.org.uk)

5 BACKGROUND AND RATIONALE

5.1 INVASIVE NON-TYPHOIDAL SALMONELLA DISEASE

Salmonella enterica is a rod-shaped Gram-negative bacterium that is further classified into approximately 2500 serovars, a number of which can cause human infection. Of these, *Salmonella* Typhi and Paratyphi are the causative agents of enteric fever and together are referred to as Typhoidal Salmonella. Non-typhoidal Salmonellae (NTS), such as *S.* Enteritidis and *S.* Typhimurium most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens. However, these organisms can also cause an invasive syndrome with bacteraemia, high fevers and metastatic infection which if untreated can lead to septicaemia and death. Invasive non typhoidal *Salmonella* (iNTS) infections are more common in resource poor settings of sub-Saharan Africa, children, the elderly and in the immunosuppressed, including HIV-infected individuals. Unlike *S.* Typhi and *S.* Paratyphi, whose only reservoir is humans, NTS can be acquired from multiple animal reservoirs including domestically farmed animals[1–3]. However, data from sub-saharan Africa appears to suggest that human-to-human transmission remains the primary mode of dissemination[4].

5.2 BURDEN OF DISEASE

The Global Burden of Disease Study estimates 535,000 annual global cases of iNTS; associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella*[5].

In the UK, NTS infection is a frequent cause of foodborne gastroenteritis outbreaks. Public Health England surveillance data showed that NTS was isolated from 8630 patient samples in 2016[6]. Whilst there are no human seroprevalence studies, it is clear that NTS remains endemic; particularly in livestock and poultry, with multiple veterinary programs implementing control methods such as testing and vaccination[7]. Although less common than in other parts of the world, invasive syndromes do also occur in the UK. Between 2004-2015, there were 2484 iNTS blood isolates in England, with neonates, individuals aged over 65 and men more likely to have a bacteraemia[8]. A case series of 82 iNTS blood cultures at a single site in the UK, found bacteraemias without an intestinal focus to be related to underlying immunosuppression in 80% of cases[9].

By far the highest burden of iNTS disease throughout the world remains in sub-Saharan Africa with 78.8% of global cases[5]. However, this is a likely underestimate of the true burden of disease, given the limited availability of diagnostics (blood cultures) in this setting. In addition, the clinical presentation of invasive disease in children is poorly defined, typically presenting as a febrile illness similar to malaria, enteric fever or pneumonia. Nevertheless, iNTS remains one of the most commonly identified causes of bacteraemia in the region[3]. A study in Malawi focused on adult medical admissions found iNTS to be the most common isolate from blood cultures, contributing to 37% of all bacteraemias in a 12 month period, and associated with a 33% case fatality rate[10]. A study in Ghana in a paediatric population found that 23.5% of bacteraemias were associated with *Salmonella* sp. of which 59% were associated with *Salmonella* Enteritidis[11]. Another paediatric single-centre study in Malawi found a case fatality rate of 20% in invasive *Salmonella* disease, of which 94% was associated with iNTS. The highest incidence was in the under three age group with 81% of cases, with a median age of 16 months[12].

The age at which infection occurs show a bimodal distribution in most African studies[2,13]. The majority of infections are in the under 5 age group with 68.3% of cases[13]; with a second peak in 30-40 years age group, which is believed to be associated with the higher incidence of HIV, malaria and malnutrition[2,13]. The Global Burden of Disease estimates a disproportionably high incidence of approximately 233,400 cases, including 31,630 deaths in the under 5 year olds globally in 2017[5].

In a meta-analysis of 22 African studies of all ages, *Salmonella* sp. was the most prevalent isolate in blood cultures at 29.4 %, with iNTS accounting for 58.4%. Of those patients who had additional HIV testing, iNTS was particularly associated with HIV infection (OR 8.2) compared with *Salmonella* Typhi bacteraemia (OR 0.07)[14]. In addition, studies have shown a decline in iNTS to be associated with a reduction in HIV, as a result of the availability of ART, as well as public health measures designed to minimise the impact of malaria and malnutrition[3,15,16]. *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) represent the most common serovars to cause invasive disease both globally and within sub-Saharan Africa. *Salmonella* Typhimurium isolates with the multi-locus sequence type (MLST) ST313 have emerged as a dominant subtype in sub-Saharan Africa. Genomic analysis of these isolates reveals genomic degradation and pseudogene formation, characteristics consistent with host adaptation and restriction by the bacterium. Along with enhanced invasive and virulence factors, the ST313 subtype is associated with multidrug resistance, potentially contributing to higher incidence and mortality of iNTS in sub-Saharan Africa[3,17]. In contrast, UK isolates are usually MLST ST19 and are commonly associated with gastroenteritis.

5.3 IMMUNO-PATHOGENESIS

It remains unclear why some infections result in local self-limiting disease whilst others cause invasive disease. In adults, iNTS is more common in the immunocompromised, including individuals with HIV, malaria, malnutrition, sickle cell disease and other immunodeficiency states, such as chronic granulomatous disease[2,3].

In children under 5 years of age, acquisition of functional antibody is associated with protection from *Salmonella* infection. Thus the highest peak of infection is between 6 months to 2 years of age, with the incidence declining thereafter. This observation correlates with protection from placentally transferred maternal antibody waning by 6 months and the development of detectable functional antibodies from natural exposure by 16 months of age[18,19]. A study in Malawi found detectable functional antibodies peaked at 35 months. In addition, this study found STm-specific CD4 T helper cells peaked at 14 months then declined, suggesting CD4 T helper cells alone were not protective.

Functional antibodies targeting iNTS can effect bacterial killing though activation of the classical pathway of complement and subsequent membrane attack complex assembly or by opsonisation, facilitating phagocytosis and oxidative burst-mediated intracellular killing. Possible targets for functional antibody include the O antigen (OAg) component of outer membrane lipopolysaccharide (LPS) or outer membrane protein antigens (OMP Ag) or flagella antigen (FliC). Human studies suggest that bactericidal activity correlated with the detectable levels of IgG to Lipopolysaccharide (LPS) but not to outer membrane protein (OMP) or flagella proteins. However antibodies against the FliC flagellar protein and membrane-bound porin proteins such as OmpD have been shown to be protective in mouse models[20,21].

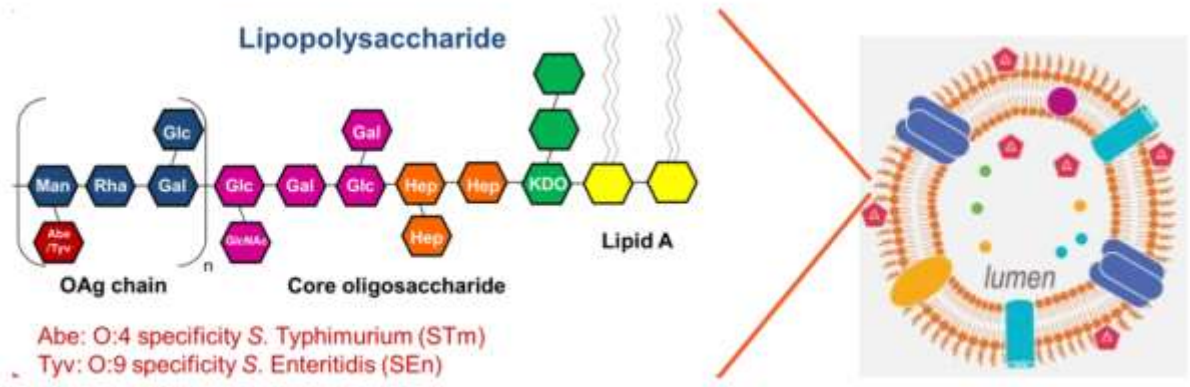


Figure 1.1 Diagram of O Antigen and Lipopolysaccharide constituent of GMMA (taken from Koeberling et al., 2017[22])

Together with lipid A, OAg forms part of the lipopolysaccharide attached to the outer membrane of Salmonella. OAg is the most immunodominant part of LPS and functions as a virulence factor with multiple studies showing attenuation of virulence in bacteria with mutations impairing OAg synthesis and longer OAg chains associated with increased resistance to antibody and complement mediated killing[23]. OAg structure can vary dependant on the individual oligosaccharide units, and this forms the basis of serotyping commonly used in microbiology laboratories. STm is associated with O:4,5 whilst SEn is associated with O:9[24].

5.4 PRIOR EXPERIENCE WITH iNTS VACCINES

The WT05 vaccine produced by Microscience Ltd. is the only iNTS vaccine candidate to date that has progressed to a phase one clinical trial. This dose escalation study randomised participants to receive either an oral attenuated *Salmonella* Typhi or an oral attenuated *Salmonella* Typhimurium at a dose 10^7, 10^8 or 10^9 CFU. Both strains were attenuated with mutations in aroC and ssaV, with ssaV associated with a reduction in function of the type III secretion system. Of the nine participants who received the *Salmonella* Typhimurium oral vaccine only the three participants in the 10^9 group had a significant antibody response[25]. There were no serious adverse events observed and each dose level of oral attenuated Salmonella *Typhimurium* appeared well tolerated. Other vaccines in development include an oral live attenuated vaccine (CVD 1931, CVD 1994), a bivalent OAg conjugate vaccine (COPS, flagellin) and protein vaccines (flagella and OmpD). All remain in the pre-clinical phase[26,27].

5.5 RATIONALE FOR VACCINE BASED ON GMMA TECHNOLOGY

The outer membrane of Gram-negative bacteria such as *Salmonella* naturally release outer membrane vesicles (OMVs) containing outer membrane proteins. OMVs have clear potential as vaccines, displaying immunogenic surface antigens in their natural conformation whilst avoiding the risks of potential infection associated with the use of live attenuated vaccines. Detergent-extracted OMVs from homogenised bacteria have been successfully licensed and used as vaccines against capsular group B meningococcal infections (MenBvac, Bexsero, VA-MENGOC-BC).

In contrast to traditional OMVs, the GMMA-technology derives outer membrane exosomes by induction of hyper blebbing from viable genetically modified bacteria. iNTS GMMA producing

vaccine strains are created from wild-type strains by deletion of *tolR*, *msbB* and *pagP* genes from *S.* Typhimurium and *S.* Enteritidis. Together, these modifications facilitate increased production of membrane blebs (Δ*tolR*) and reduced-acylation of the lipid A component of bacterial lipopolysaccharide (LPS via Δ*msbB* and Δ*pagP*). GMMA particles with hexa- or penta-acylated LPS induce less cytokine production *in vitro* by human peripheral blood monocytes compared to wild type GMMAs (containing hepta-acyltaled lipid A), which potentially reduces *in vivo* reactogenicity. Furthermore, GMMAs are potentially highly immunogenic, as they present O polysaccharide (OAg) and outer membrane protein antigens identified as immune targets in their native configuration, with minimal expression of cytoplasmic or inner membrane proteins. Purified GMMA particles are filtered, concentrated and implemented as a vaccine[28,29].

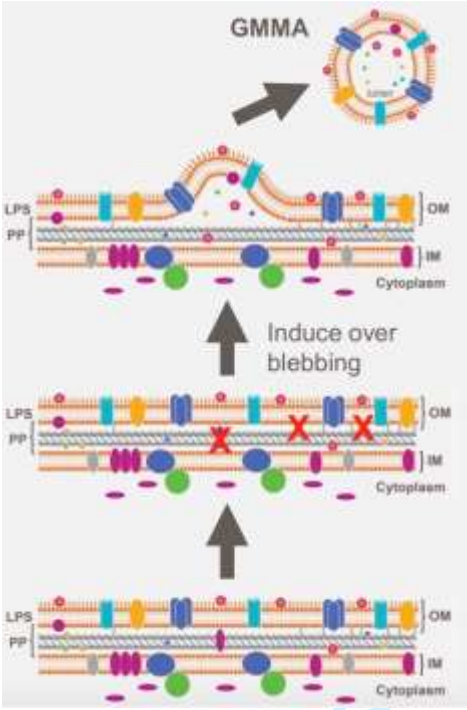


Fig 1.2 Outer membrane blebbing to create GMMA particles (taken from Koeberling 2017[22])

5.6 CLINICAL STUDIES OF ENTERIC GMMA VACCINES

This is the first trial to investigate a 2-component iNTS GMMA vaccine in humans. However, there have been four proof-of-concept clinical trials of a *Shigella* Sonnei GMMA vaccine; three phase 1 trials (including one phase 1 extension trial) in Europe[30,31] and one phase 2a trial in Kenya[32]. Over 100 participants have been enrolled, with all studies demonstrating a well-tolerated vaccine with an acceptable safety profile. The most common adverse event was mild to moderate injection site pain. A transient asymptomatic neutropenia was reported in 9.6% of participants in the two initial phase 1 European trials[30].

In vitro analysis of *Shigella* GMMA have revealed they contain > 95% of the outer membrane and periplasmic proteins[33]. By extrapolation this suggests the iNTS GMMA vaccine may contain a similar percentage of proteins which may contain multiple antigens including OAg and OMP Ag eliciting a potentially broadly protective immune response.

In Phase I trials, the *Shigella* GMMA vaccine used a 3-dose regime with intervals of 0, 1 and 2 months. The iNTS GMMA vaccine will use a 3-dose regimen at 0, 2 and 6 months to allow involution of B cell germinal centres and T cell responses between doses to better investigate

booster and memory responses. The highest *Shigella* GMMA vaccine dose so far tested contained approximately 6 µg OAg and 100 µg protein, while the highest iNTS GMMA dose will contain 40 µg OAg and approximately 31 µg protein.

THE INVESTIGATIONAL PRODUCT: 2-COMPONENT iNTS GMMA SALMONELLA VACCINE

5.7 DESCRIPTION OF INVESTIGATIONAL PRODUCT

The iNTS GMMA Vaccine consists of the outer membrane exosomes of the two most common serovars causing invasive disease, *Salmonella* Enteritidis (O:9) and S*almonella* Typhimurium (O:4.5), adsorbed to Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline pH 6.5. Two doses levels will be used for this study: a full dose of 20 µg STmGMMA as OAg and 20 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml full dose; and a lower dose at 3.8 dilution rendering a vaccine consisting of 5.3 µg STmGMMA as OAg and 5.3 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml dose. The placebo matches the vaccine matrix and consists of Alhydrogel (0.35 mg AL^{3+} / 0.5 mL dose) in isotonic 20mM Phosphate buffered saline pH 6.5 without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

5.8 CHARACTERISTICS OF VACCINE PREPARATION

STmGMMA and SEnGMMA, the iNTS vaccine active components, are formulated separately at 80 µg GMMA as OAg/ml batches with the GMMA absorbed onto 0.7mg Alhydrogel as Al^{3+}/ml Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline at pH6.5 . Each batch is aliquoted into vials containing 0.7 ml of either STmGMMA/ Alhydrogel or SEnGMMA Alhydrogel, stored at 2-8 ºC.

At the clinical site, 0.5ml of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel will be mixed in an empty vial using a standard operating procedure to yield 1ml of the 2-component iNTS GMMA vaccine containing 40 µg STmGMMA as OAg and 40 µg SEnGMMA as OAg and 0.7 mg Al^{3+} of Alhydrogel suspended in isotonic 20mM Phosphate buffered saline pH 6.5. This procedure including dilution for the lower dose iNT-GMMA vaccine is detailed in the clinical study plan and will be performed at the clinical site by trained study personnel. This procedure has been developed and evaluated by GVGH. Further stability testing has been performed to confirm the quality of the iNTS-GMMA vaccine for up to 6 hours post mixing, however it is envisaged that the final iNTS-GMMA vaccine will be used within 1 hour of mixing.

5.9 IMMUNOGENICITY IN MICE

Anti-OAg ELISA titres correlate with functional antibody assays consistent with immunity in children[18,19]. Mouse studies using challenge with either wild type *Salmonella* Typhimurium or with an OAg knockout strain higher levels of functional bactericidal antibodies in animals exposed to the bacteria expressing OAg [24].

Mice immunised either with STm and SEn GMMA vaccine showed both an elevated OAg titre by ELISA and complement mediated antibody killing via serum bactericidal assay (SBA). These results were then replicated in mice immunised with the two component (STm and SEn) iNTS GMMA vaccine (Figure 1.3) confirming the immunogenicity and validity of the bivalent

vaccine[34]. On challenge with either live *S.* Typhimurium or *S.* Enteritidis, immunised mice showed a reduction of bacterial burden (CFUs) in the spleen and liver.

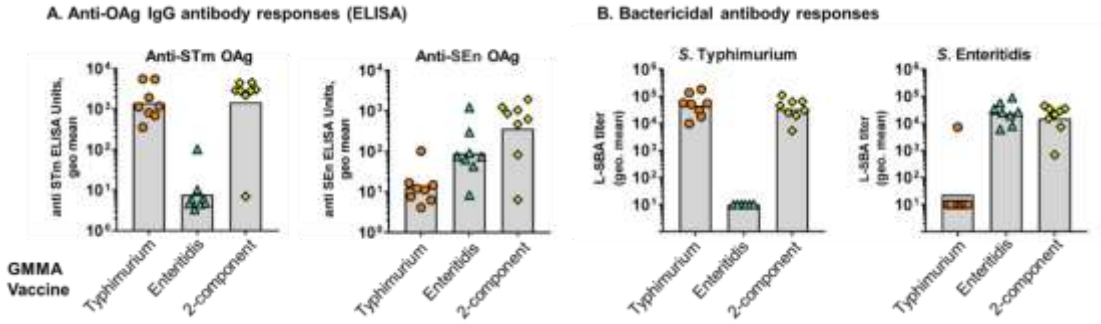


Figure 1.3 (A) Anti-STm and anti-SEn OAg IgG serum antibody responses (ELISA units) and (B) SBA responses against *S.* Typhimurium and *S.* Enteritidis strains. CD1 mice were immunized twice, four weeks apart with 0.16 µg (based on OAg) STmGMMA or SEnGMMA adsorbed on Alhydrogel or a mixture of 0.16 µg each of the two formulated GMMA (2-component iNTS-GMMA). Sera were collected two weeks after the second immunization. Symbols represent results from individual mice, bars represent group geometric mean titres.

In addition there was no significant difference in antibody response when the GMMAs were combined with Alhydrogel[34]

Study	Vaccine	Adsorbent	Dose [µg]	Anti-OAg IgG ELISA titre (geometric mean)
1	STmGMMA	None	2.5	61588
	STmGMMA	Alhydrogel	2.5	69572
2	SEnGMMA	None	2.5	8953
	SEnGMMA	Alhydrogel	2.5	10401

Table 1.1 Anti-OAg IgG ELISA units induced in mice by STmGMMA and SEnGMMA administered with or without Alhydrogel

5.10 IMMUNOGENICITY IN RABBITS

OAg is also a immune target in rabbits. Animals challenged with wild type *Salmonella* Typhimurium or OAg knockout strains develop higher bactericidal antibody response in the wild type arm[24].

In a rabbit immunogenicity study, STm GMMA/Alhydrogel, SEn GMMA/Alhydrogel and the 2-component iNTS-GMMA vaccine were well tolerated and induced strong anti-STm OAg and anti-Sen OAg serum IgG responses and high bactericidal antibody activity against *S.* Typhimurium and *S.* Enteritidis strains.

5.11 TOXICOLOGY

The dosing regimen has been estimated based on immunogenicity in mice and rabbits[24,34], in conjunction with the results of monocyte activation, pyrogenicity and repeat dose toxicology studies in rabbits.

In order to maintain immunogenicity whilst reducing reactogenicity, expression of Lipid A, a potent stimulator of the innate immune system, has been modified via deletions of the genes msbB and pagP. A monocyte activation model with human PBMC stimulated by a mixture of 1:1

STm and SEn iNTS GMMAs produced less IL-6 (a marker of inflammation) than wild-type GMMAs[24]. A similar cytokine release profile was stimulated by unformulated *S. sonnei* 1790-GMMA, which, as Alhydrogel formulated vaccine, were well tolerated in EU and Kenyan adults.

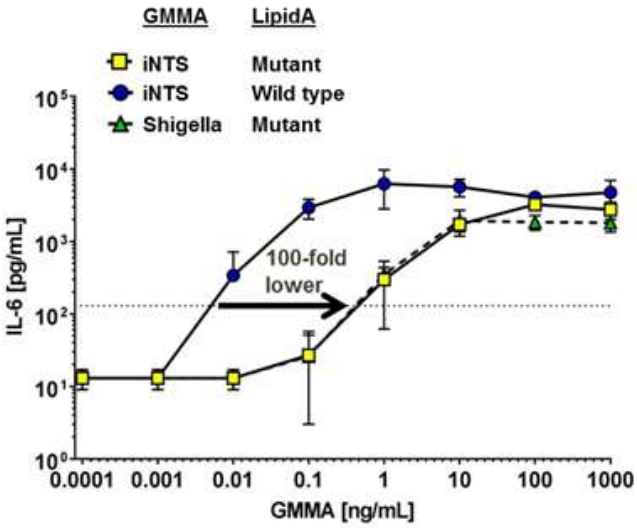


Figure 1.4 Monocyte activation test IL-6 response after incubation with different concentrations of a 1:1 mixture of STmGMMA and SEnGMMA in comparison to *S. sonnei* 1790-GMMA

A rabbit pyrogenicity study was performed using intramuscular administration of undiluted single GMMA formulations and 2-component iNTS-GMMA vaccine. The 2-component iNTS-GMMA vaccine showed a mean peak temperature rise of 1.1^{O}C using the highest target human iNTS dose. This mean peak temperature rise was 0.8 ^{O}C less than the mean peak temperature rise without Alhydrogel, suggesting whilst there is minimal change in immunogenicity, reactogenicity is reduced with the addition of Alhydrogel.

	Group mean initial temperature [°C]	Group mean maximum temperature [°C] with time indicated	Group mean temperature [°C] at end of measurement [5 hours after vaccination]	Mean peak temperature time point after vaccination [minutes]	Mean peak temperature rise [°C]
iNTS-GMMA (STmGMMA/Alhydrogel + SEnGMMA/Alhydrogel) 20 µg + 20 µg [OAg]	38.6	39.7	39.6	210	1.1
Unformulated mixture of STmGMMA + SEnGMMA 2 µg + 2 µg [OAg]	39.3	41.2	39.8	180	1.9

Table 1.2 STn+SEn with or without Alhydrogel dosed rabbits, showing a mean temperature rise of 1.1 ^{O}C in STm/Sen with Alhydrogel (iNTS Vaccine)

A repeat dose toxicology study in rabbits has been performed using the highest anticipated human iNTS dose at Covance Laboratories (Harrogate, UK).

Group number	Group description	Dose level (µg GMMA [OAg])	Dose Days	Animal numbers			
				Main necropsy		Recovery necropsy	
1	Control (Saline)	0	1, 15, 29, 43	5 Male	5 Female	4** Male	5 Female
2	Test	20 + 20*	1, 15, 29, 43	5 Male	5 Female	5 Male	5 Female
*The test article was 0.5 mL of a bed-side mixing of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel each containing 20 µg of each GMMA (quantified based on OAg) and 0.35 mg Al^{3+} Alhydrogel **One animal was withdrawn during the pre-immunization phase							
Main necropsy: Day 46 (3 days after last injection) Recovery necropsy: Day 71 (28 days after last injection)							

Table 1.3 Outline of repeat dose toxicology study in rabbits. INTS GMMA vaccine versus Saline Control Group

New Zealand White rabbits were grouped into a test and control (saline) group. They then received 0.5ml (40µg OAg) of the iNTS-GMMA vaccine at 2 weekly intervals. The results indicated a systemically well-tolerated iNTS GMMA vaccine. Please see the Investigator's Brochure for further details.

5.12 RATIONALE / AIM OF TRIAL

Thirty to forty-two participants in the 18-55 year age group will be recruited into the trial. The trial will follow a dose escalation design with the first group randomised 1:1 to receive a lower dose of 10.6 µg total OAg of iNTS-GMMA vaccine (5.3 µg of STmGMMA as OAg and 5.3 µg of SEnGMMA as OAg) or placebo. A second group will be randomised 1:1 to receive the full intended dose of 40 µg total OAg of iNTS-GMMA (20 µg of STmGMMA as OAg and 20 µg of SEnGMMA as OAg) or placebo. A third group will be randomised 2:1 to receive the iNTS-GMMA vaccine at intended full dose versus a placebo (See 7.Trial Design). An adequate safety review from the Data Safety Monitoring Committee (DMSC) will allow progression of the trial from group 1 to group 2 and from group 2 to group 3. Given there are no current licensed iNTS vaccines, the comparator for this trial will be an Alhydrogel placebo. The last dose of the vaccine will be on Day 168, with a final visit 6 months thereafter. Thus, participants will remain on the trial for 12 months in total.

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine. The immunogenicity of the vaccine will be assessed by OAg ELISA and serum bactericidal assays specific to each vaccine serovar. Additional exploratory immunological assays assessing functional antibody (including but not limited to opsonophagocytosis and glycosylation), B cell and T cell responses will be performed. If the results of this trial indicate a vaccine with an adequate safety profile, progression to a further phase 1 clinical study in an iNTS endemic country will occur.

6 OBJECTIVES AND OUTCOME MEASURES

6.1 PRIMARY OBJECTIVE

To determine the safety and tolerability between two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.2 SECONDARY OBJECTIVE

To investigate the immunogenicity at two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.3 EXPLORATORY OBJECTIVES

To further investigate the immunogenicity using exploratory immunological analyses of the two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals

6.4 PRIMARY ENDPOINTS / OUTCOME MEASURES

The recording and assessment of local and systemic adverse events following administration of each vaccine dose;

- Tenderness and pain at the injection site
- Induration
- Redness

- Swelling
- Headache
- Malaise
- Myalgia
- Nausea and/or vomiting
- Diarrhoea
- Abdominal Pain
- Anorexia
- Arthralgia
- Fatigue
- Fever
- Blood parameters (haematology / biochemistry)
- Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR

6.5 SECONDARY ENDPOINTS / OUTCOME MEASURES

Immunological assays to study immune responses to vaccines, including:

1. Antibody concentration against serovar specific O antigens determined by enzyme linked immunosorbent assay (ELISA) before and after each dose.

6.6 EXPLORATORY ENDPOINTS / OUTCOME MEASURES

Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to:

1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose .
2. Serum bactericidal antibody (SBA) titres against vaccine homologous strains before and after each dose
3. Serum bactericidal antibody (SBA) titre isogenic strains before and after each dose
4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose
5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose
6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose
7. Transcriptomic profile analysis after immunization to investigate differential expression of innate, B cell and T cell activation gene modules and DNA storage for investigation of the genetic associations with the immune response
8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays

10. Faecal antibody concentration against O antigen determined by by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection.
11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.

7 TRIAL DESIGN

7.1 OVERVIEW OF TRIAL DESIGN

This is a first in human, phase 1, single-centre participant-observer blind study to assess the safety and immunogenicity of three administrations of iNTS-GMMA vaccine in healthy adults. Participants will be considered enrolled in the study once their first vaccination has been administered. The total number of participants is 30-42, and individuals will be divided into 3 groups as described below:

1. **<u>Group 1</u> (PARTICIPANT-OBSERVER BLIND)** - lower dose 10.6 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or a placebo according to the study plan outlined in section 7.2.1. A favourable DSMC review of the safety data from this arm will be required before commencement of group 2. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

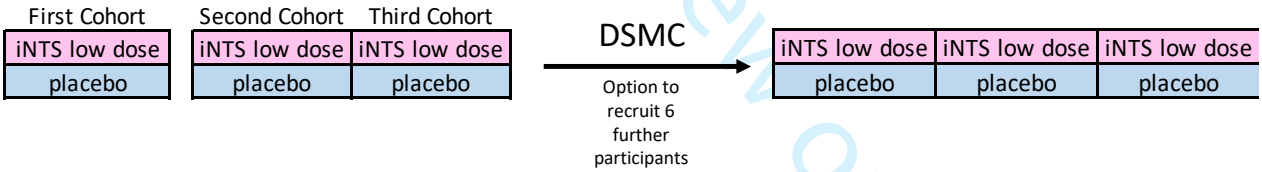


Figure 2.1 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive lower dose iNTS-GMMA vaccine or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

2. **<u>Group 2</u> (PARTICIPANT-OBSERVER BLIND)** - full dose 40 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will only proceed after DSMC review and approval of Group 1. This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 40 µg total OAg of iNTS GMMA vaccine or a placebo. See section 7.2.1 for further details regarding safety and dose escalation. A favourable DSMC review of the safety data from this arm will be required before the commencement of group 3. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

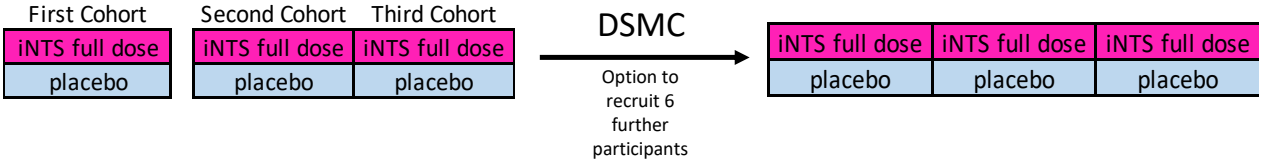


Figure 2.2 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive iNTS full dose or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

3. **Group 3 (PARTICIPANT-OBSERVER BLIND)** – 18 participants will be randomised 2:1 to receive three administrations at D0, D56 and D168 of either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or a placebo. Recruitment to this group will be subject to a favourable interim review of the safety data of group 2 by the DSMC.

   Depending on DSMC review and participant numbers in Group 1 and 2, 18 participants will be enrolled into Group 3:
   Either:

iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.3 Eighteen Participants, randomised 2:1 to receive full dose iNTS-GMMA vaccine or placebo

Or:

iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.4 Eighteen Participants, randomised 2:1 to receive lower dose iNTS-GMMA vaccine or placebo

7.2 DOSE ESCALATION

As this is a first in human trial, we will operate a dose escalation policy in groups 1-3 between lower and full doses. The rationale for these doses is based on experience from animal toxicology studies (5.11 TOXICOLOGY). Escalation between these doses will be dependent on a favourable safety review by the DSMC. This unblinded safety review will consist of AEs from all participants vaccinated in group 1, 2 and 3 at the DSMC review time points indicated below. Group 1 and 2 must have a minimum of 6 participants vaccinated in each group prior to DSMC review. AE Safety Data will include solicited, unsolicited, observation related, laboratory, SAE / SUSARS. Note SAE/SUSARs have specific DSMC reporting instructions as outlined in Section 11.6.

7.2.1 DOSE ESCALATION PROCESS FOLLOWING D0 VACCINE

Group 1 – Lower dose IMP

Initially, 6 participants will be subdivided into 3 pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or placebo. Initially

only one pair will be vaccinated and observed for any adverse reactions for 48 hours before further participants are vaccinated. The medically qualified investigator will be asked to provide the decision on whether to proceed after a blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a second and third pair will be vaccinated at least one hour apart. This will bring the total number of participants in this group to 6, of which 3 receive the lower dose IMP, and 3 receive the placebo.

Group 1: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 1. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same lower dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 2 will proceed.

If participants in Group 1 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 2 – Full dose IMP

In the full dose group, 6 participants will initially be subdivided into 3 pairs as in group 1. Within each pair the two participants will be randomised 1:1 to receive the full dose 40 µg total OAg of iNTS-GMMA vaccine or placebo. Initially only one pair will be vaccinated and observed for any adverse reactions/events for 48 hours before more volunteers are vaccinated. The medically-qualified investigator will be asked to provide the decision on whether to proceed after blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a further two paired cohorts will be vaccinated with the 40 µg total OAg dose. This will bring the total number of participants in this group to 6, of which 3 receive the full dose IMP, and 3 receive the placebo. The second and third pairs will be vaccinated at least one hour apart.

Group 2: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 2. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same full dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 3 will proceed.

If a favourable opinion is given for Group 1, but not for Group 2, then the DSMC may allow the ‘lower dose’ to be used in Group 3 instead of the full dose. If favourable decision is received

from the DSMC a substantial amendment would be required to continue further vaccination clearly stating the iNTS-GMMA vaccine dose to be administered.

If participants in Group 2 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 3 – Low or Full Dose

In this group, 18 participants will be randomised 2:1 to receive the iNTS GMMA vaccine (either the lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo. The total number of participants in the study will be between 30-42 individuals, depending on the final numbers of participants recruited to group 1 and 2.

7.3 DSMC (Unblinded) REVIEWS

The DSMC will review the unblinded safety data at two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

7.4 GROUP ALLOCATION

Allocation to each group will be decided by order of enrolment into the trial. Participants will be considered enrolled in the trial once they receive their first vaccination. Groups 1, then 2 will be preferentially recruited to ensure study progress as per the dose escalation process above with randomisation within each cohort pair. Once safety data have been reviewed by the DSMC, participants recruited to group 3 will be randomised to receive either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo in a 2:1 ratio.

7.5 SAFETY MONITORING

Safety outcomes of the participants will be monitored throughout the study. This will be done by monitoring symptoms at visits, daily review of an electronic symptom diary up to D6 after every vaccine received on the study, and safety blood tests (Table 2.1).

Group 1 and 2 vaccinations will proceed in a staggered fashion (Fig 2.2), dependent on safety review by the DSMC. The time interval between the 7 days post the last participant to receive their first vaccine administration in Group 1 to the first participant in Group 2 to receive their first vaccine administration therefore allows continuous monitoring of the safety of both the second (D56) and third (D168) vaccinations in Group 1 participants receiving low dose IMP prior to the respective vaccinations in Groups 2.

The medically qualified investigator will be asked to provide the decision on whether to proceed to second (D56) and third (D168) vaccine administration in Group 2 after reviewing 7 days of blinded safety data after the last participant receives the second and third vaccine administrations in Group 1 respectively (See section 11.14). This review will consider blinded safety data from

the results of participant e-diaries, eCRFs, AEs after vaccination and safety bloods. This review will be based on the clinical judgement of the investigator with the option to escalate to the DSMC if any concerns. A similar review will be conducted using the safety data from the last participant to receive their second (D56) and third vaccine (D168) administrations of Group 2 prior to proceeding the the second (D56) and third (D168) vaccine administration of Group 3.

Cumulative toxicity has not been observed with similar GMMA based *Shigella* vaccines and is not expected with this vaccine. Thus staggered vaccination of cohorts of second and third vaccines in Group 1 and 2 will not be performed.

Full details about the reporting of any adverse events or serious adverse events, and the role of the DSMC beyond the dose escalation reviews is discussed in section 11 of the protocol.

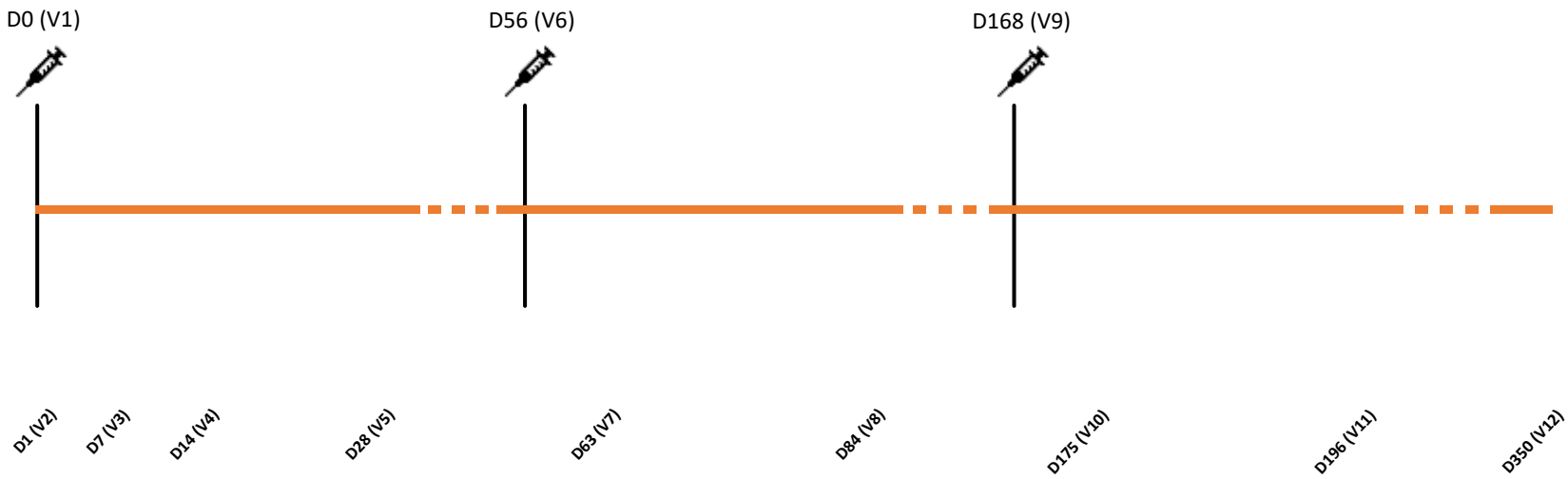
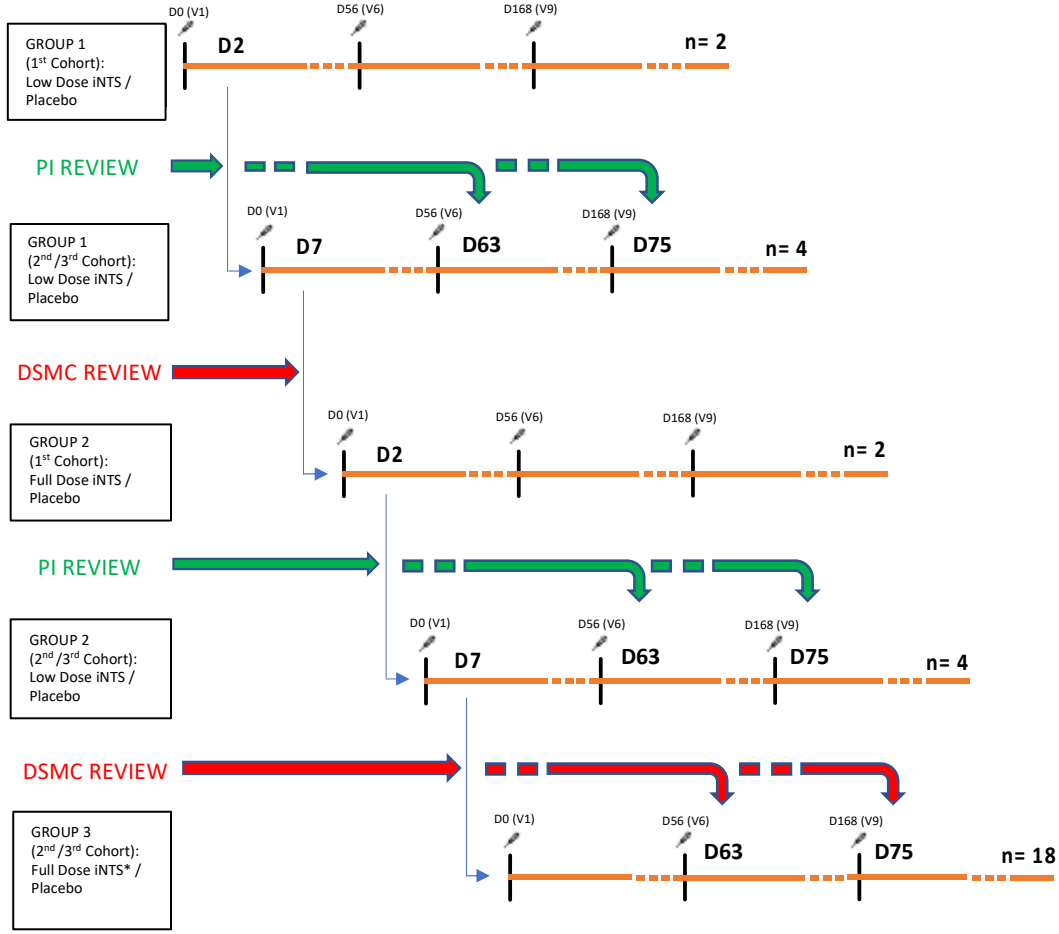


Figure 2.1 Vaccine schedule: Groups 1 – 3



* Low Dose iNTS may be considered if unacceptable safety signal in Group 2 high dose iNTS following Investigator and DSMC review

Figure 2.2 Dose Escalation Schedule: Groups 1 - 3

	Screening	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Indicative Study Day		D0	D1	D7	D14	D28	D56	D63	D84	D168	D175	D196	D350
Day post last vaccine		0	1	7	14	28	56	7	28	112	7	28	182
Visit Window (days)	V1 - 90*		0	+/- 1	+/- 2	+/- 7	+/- 4	+/- 1	+/- 7	+/- 4	+/- 1	+/- 7	+/- 28
Informed consent	x												
Confirmation of eligibilty criteria		x					x			x			
Obtain 24 hr contact details		x					x			x			
Medical history (including demographics and medication)	x												
Interim medical history (including concurrent medication)		x	x	x	x	x	x	x	x	x	x	x	x
Review / collection of AEs and SAEs since last visit		x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x												
Vital signs	x	x	x	x	x	x	x	x	x	x	x	x	x
Urine pregnancy test	x	x					x			x			
Urine dipstick (with urine analysis as needed)	x												
Blood sample	x	x	x	x	x	x	x	x	x	x	x	x	x
Oral Fluid Swab		x			x	x	x	x	x	x	x	x	x
Stool/Faecal Sample (Optional Only)		x										x	x
Vaccination		**x**					**x**			x			
e-Diary entries		x	x	x			x	x		x	x		
Intervention arm allocation		x											

Table 2.1 Visit Structure: Groups 1 - 3

Participants will complete the scheduled visits within the visit windows outlined above, however due to unforeseen circumstances such as participant unavailability, the visit may still proceed outside of window if reasonable to do so, as judged by the investigator. A physical exam is performed at screening. A physical exam is not routinely performed at the remaining visits unless the clinical history or situation deems it necessary.

*Once the screening visit has been completed, V1 must be scheduled within a maximum of 90 days of the date of the screening visit. This interval allows for receipt of GP medical summaries or repeat blood tests. If the V1 for the potential participant falls outside of this period, a repeat screening visit must be performed if the participant remains eligible and willing. However certain tasks which are unnecessary to be repeated as judged by the investigator may be brought forward. Tasks which must be repeated at this re-screening visit include: consent, interim medical history and urine pregnancy test (if applicable). Other tasks may also be performed at the Investigator's discretion.

8 PARTICIPANT IDENTIFICATION

8.1 TRIAL PARTICIPANTS

Male or female participants aged 18-55 years inclusive who are in good health (as determined by a study doctor) and who are able to provide written informed consent, will be eligible for inclusion in this study. Between 30-42 participants are required.

8.2 INCLUSION CRITERIA

Participants must satisfy all of the following criteria to be considered eligible for the study:

- Willing and able to give informed consent for participation in the study
- Aged between 18 and 55 years inclusive
- In good health as determined by
    - Medical history
    - Physical examination
    - Clinical judgment of the investigators
- (Females) Willing to use highly effective contraception as defined in Section 8.5 from one month prior to receiving the first vaccine and for the duration of the study
- Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
- Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study.
- Willing to allow study team access to medical records for the purposes of eligibility assessment and / or safety follow up during the trial.
- Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS).

8.3 EXCLUSION CRITERIA

The participant may not enter the study if any of the following apply:

- History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators. This includes any history of **significant** disease in the following:
    - Cardiovascular disease including congenital heart disease, previous myocardial infarction, valvular heart disease (or history of rheumatic fever), previous bacterial endocarditis, history of cardiac surgery (including pacemaker insertion), personal or family history of cardiomyopathy or sudden adult death
    - Respiratory disease such as uncontrolled asthma and chronic obstructive pulmonary disease
    - Endocrine disorders such as diabetes mellitus and Addison's disease
    - Significant renal or bladder disease
    - Biliary tract disease

    - Gastro-intestinal disease such as inflammatory bowel disease, abdominal surgery within the last two years, coeliac disease and liver disease (including hepatitis B or C infection)
    - Neurological disease such as seizures and myasthenia gravis
    - Haematological disease including coagulation problems
    - Metabolic disease such as glucose-6-phosphate dehydrogenase deficiency
    - Psychiatric illness requiring hospitalisation
    - Depression, anxiety or other psychiatric illness whose severity is deemed clinically significant by the study investigators
    - Known or suspected drug and/or alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week)
    - Non-benign cancer, except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ
- Have any known or suspected impairment or alteration of immune function, resulting from, for example:
    - Congenital or acquired immunodeficiency (including IgA deficiency)
    - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
    - Autoimmune disease
    - Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy (including for more than 7 days consecutively within the previous 3 months).
- Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing (at the discretion of an Investigator) within the recruitment timeline of the study
- Have received any oral typhoid vaccination (e.g. Ty21a or M01ZH09) within the last 3 years or a paratyphoid vaccine (as part of a clinical trial)
- Have participated in previous typhoid or paratyphoid challenge studies (with ingestion of challenge agent).
- Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
- Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (COVID-19 vaccine exempt, see Section 9.14)
- Any history of allergy or anaphylaxis to a previous vaccine or vaccine component
- Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
- Participation in another research study involving an investigational product or that which may compromise the integrity of the study (e.g. significant volumes of blood already taken in previous study) in the past 12 weeks, or are planning to do so within the trial period
- Planned donation of blood/blood products outside of the study and during the trial period.
- Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
- Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study[1]

[1] As defined by CTFG Recommendations related to contraception and pregnancy testing in clinical trials, current document: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf [accessed 23rd March 2022]

- Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m^2
- Any other significant disease or disorder which, in the opinion of the Investigator, may:
  - Put the participants at risk because of participation in the study
  - Influence the result of the study
  - Impair the participant's ability to participate in the study

8.4 TEMPORARY EXCLUSION CRITERIA

The following applies to both **initial enrolment** and **subsequent vaccination** visits. If the temporary exclusion resolves within the time constraints of the trial, they can be enrolled and/or progression in the trial can continue.

- Receipt of any systemic corticosteroid (or equivalent) treatment within 14 days prior to vaccination, or for more than 7 days consecutively within the previous 3 months
- Febrile illness (oral temperature ≥37.5°C) or systemically unwell on the day of vaccination
- If a participant is taking systemic antibiotics then the vaccination is postponed until 7 days after the last dose. This does not apply to topical antibiotic preparations
- Use of antipyretics in the 4 hours prior to vaccination
- A laboratory AE considered, in the opinion of the Investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered
- Symptoms of COVID-19, without confirmation of infection (as per current government guidelines) 14 days prior to vaccination visit
- Validated positive (first of episode) SARS-CoV-2 test (NAAT or antigen) within 4 weeks prior to vaccination visit
- Any illness / AE considered, in the opinion of the investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered

8.5 PREGNANCY AND CONTRACEPTION

The possible adverse effects of the iNTS-GMMA vaccine on the outcome of pregnancy are unknown; therefore, pregnant women will be excluded from the study. Women of childbearing potential will be required to use an effective contraceptive measure. Contraception should be maintained during the vaccination period and for the duration of the study. Should a volunteer become pregnant during the trial, she will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. We would not routinely perform venepuncture in a pregnant volunteer unless there is clinical need.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible. The active components of the iNTS-GMMA vaccine are the GMMA particles which consist of blebs of the outer membrane of the two Salmonella serovars, S.Enteritidis and S.Typhimurium. As a result, both components lack the necessary machinery for replication in vivo. They have been designed to stimulate an immune response to the antigen(s) contained within the GMMAs. Together with the lack of replicative machinery and a maximum iNTS-GMMA dose of 40µg makes the risk of human teratogenicity/fetotoxicity possible/unlikely.

A woman is considered of childbearing potential, i.e fertile, following menarche and until becoming post- menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophrectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient.

Female volunteers of childbearing potential are required to use a highly effective form of contraception until their last follow-up visit. Acceptable forms of contraception for female volunteers include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral/intravaginal/transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral/injectable/implantable)
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised male partner
- sexual abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

9 TRIAL PROCEDURES

9.1 RECRUITMENT

Identification of study participants

In order to recruit the required cohort of 30-42 participants, several strategies may be employed, including but not limited to:

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor's surgeries, tertiary education institutions and other public places with the permission of the owner/ proprietor.
- Direct mail-out / SMS/text message / emails: Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases. These include the National Health Applications and Infrastructure Services (NHAIS) via a NHAIS data extract or equivalent. For the NHS databases initial contact to potential participants will not be made by the study team. Instead study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd (or equivalent company), in order to preserve the confidentiality of potential participants. CFH Docmail Ltd (or equivalent company) is accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70). For mail-outs via the electoral register, we will have access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH

Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company). Volunteers may also be recruited using direct SMS/text message, or emails to potential participants identified by GPs from their databases.

- Email campaign: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and link to study website by email or hard copy.
- Oxford Vaccine Centre (OVC) database for healthy volunteers: Direct email and link to members of the public who have registered their interest in potentially volunteering for clinical trials conducted by OVC. This secure database is maintained by OVC and members of the public registered here have given consent to have their details recorded and be contacted expressly for this purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to volunteering for any trial they are contacted about.
- Media advertising: Local media, newspaper and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- Website advertising: Description of the study and copy of information booklet on the OVG website.
- Social media: Advertisements placed on OVG or University of Oxford Social media accounts or targeted social media platform advertisements including, but not restricted to, Twitter, Facebook and Instagram
- Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

Recruitment, approach and initial eligibility assessment of potential study participants
Potential participants who are interested in the study will be able to contact the OVG by telephone, email or trial website for further information. Once an expression of interest has been received by OVG, an information sheet will be sent via mail, email or downloaded from the website by potential participants to read at their leisure. If participants are willing to proceed, they will be initially screened by a website questionnaire and/or telephone before they are invited for a screening and consent visit, where their eligibility will be assessed by member of the clinical research team at the Oxford Vaccine Group. Permission to access the volunteer medical records either via the electronic records system or GP will be sought (if possible) prior to the screening visit. Participants will be asked to sign a secure electronic document (hosted by the REDCap database) which will then be counter-signed by a study team member. Alternatively written permission to access medical records will be sought at the volunteers screening and consent visit.

9.2 INFORMED CONSENT

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Consent will be sought as described in relevant SOPs.
Written and verbal versions of the participant information booklet and informed consent form will be presented to the participant, detailing no less than:

- the exact nature of and the rationale for performing the study
- implications and constraints of the protocol

- the risks and benefits involved in taking part

It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. The participant will be allowed at least 24 hours to consider the information from when they receive it, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study.

The participant will have the opportunity to discuss the study with a medically qualified investigator. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the appropriately trained and delegated clinician. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site.

Participants will be informed that they would also be eligible for BioBank ('Oxford Vaccine Centre Biobank' Southampton & South West Hampshire LREC (B) 10/H0504/25). BioBank is a separate study and optional to all participants of studies conducted by OVC. Separate consent is sought for this.

9.3 BASELINE ASSESSMENTS AT SCREENING

Once informed written consent is obtained, the following baseline assessments and information is collected as part of the assessment of inclusion/exclusion criteria:

- Participant demographics; age, sex and ethnicity
- Travel history (record travel to any country outside of the UK for longer than 2 months; record any travel to Sub-Saharan Africa or South East Asia)
- Medical history
- Contraception; female participants are asked if they are willing to use effective contraceptive measures one month prior to vaccination and for the remainder of the study
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- Recording of resting pulse, blood pressure, temperature, weight and height
- Physical examination; cardiovascular, respiratory, abdominal and gross neurological examination
- Urine dipstick (and laboratory analysis if appropriate) and urine pregnancy test
- Blood samples for: haemoglobin count, white cell indices, platelet count, serum sodium, serum potassium, serum urea, serum creatinine, liver function tests, C-reactive protein, HIV, Hepatitis B and C.
- Collect emergency contact details

The medical, vaccination and prescribed medication history are initially based on participant recall. However, with prior participant approval, patient medical summary, vaccination and prescribed medication history will be formally requested from the GP or accessed via the electronic patient record (if available) at the screening visit if not already requested or accessed in advance. In addition, all participant GPs will be notified of their participation in the study.

Consent will be taken to register the participant on The Over-volunteering Prevention System (TOPS) database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs and blood donations. This will be done using the

participant's National Insurance number or Passport Number. The TOPS database will be checked for any conflicts at screening, however formal registration will be done at enrolment.

9.4 RANDOMISATION

Groups 1 and 2 will be cohorted in pairs randomised 1:1 to receive the lower dose or full dose iNTS GMMA vaccine respectively versus placebo. Once the study has progressed to Group 3, 18 participants will be randomised 2:1 to receive the investigational product versus placebo.

Randomisation will be conducted using an electronic system within the RedCAP database. Participants in Group 1 and 2 will be randomised 1:1 in blocks of two, to meet the total numbers required for each group. Participants in Group 3 will be randomised 2:1 in blocks of three, to meet the total numbers required.

9.5 BLINDING AND CODE-BREAKING

This study will be conducted in an observer and participant blind fashion. The study blind will be maintained from the time of participant randomisation until participant unblinding which will occur once the last participant has completed their final visit.

There will be dedicated blinded and unblinded study teams. Blinded staff will include clinical study doctors (including the CI), study nurses, administrative and laboratory staff who will be directly managing participants and participant samples. Vaccine and placebo will be reconstituted, checked and administered by a dedicated unblinded study team, such that the participant will not be aware of which vaccine they have received. Assays requiring blinding in the laboratory (eg Elispots), will be measured by at least one individual blinded to vaccine or placebo allocation.

Participants and their General Practioners will receive written notification by letter or email of whether they have received the vaccine or placebo at the time of full study unblinding.

Unblinding may also occur at an earlier time point in the event of the occurrence of SAEs, SARs or SUSARs (please see section 11).

In the case of medical emergency the investigator will have direct access to unblinding of the participant(s) by opening of the participant(s) sealed envelope containing their vaccine record. This will be confirmed by electronic unblinding via the REDCap database, which can be performed in the first instance to avoid any delay in unblinding due to a medical emergency or out of hours.

This will be conducted under the guidance of the Data Safety and Monitoring Committee. Unblinding procedures will be conducted in accordance with local OVG SOPs.

9.6 VACCINATION VISITS

Vaccination visits are held at the CCVTM. The visit procedure for the vaccination visits will be as follows:

- Ensure that participant consent remains valid and confirm continued consent.

- Measure Weight, Height and Body Mass Index
- Obtain and document interim medical history since the last visit and check eligibility criteria, specifically temporary exclusion to vaccination
- Review for AEs and SAEs since the last visit
- Training on electronic diary card entry and if second or third vaccine review of diary card entries and laboratory AE profile
- Record oral temperature, pulse and blood pressure
- Perform urinary pregnancy test for females
- Perform blood draw
- Perform oral fluid swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Administer vaccine by IM injection into non-dominant deltoid muscle by the second team member in second clinic room (who remains unblinded, following iNTS-GMMA vaccine preparation as per clinical study plan)
- Observe for immediate adverse events for 60 minutes, followed by post vaccine checks including routine observations, review of vaccine site and assessment of wellbeing
- Schedule next visit and re-iterate participant requirements such as return of the Diary Card entries

9.7 NON-VACCINATION VISITS

Other visits may require the following procedures:

- Obtain and document interim medical history since screening and check continued eligibility
- Review for AEs and SAEs since the last visit
- Review eDiary entries and laboratory blood tests
- Record oral temperature, pulse and blood pressure
- Perform blood draw
- Perform oral swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Schedule next visit and re-iterate participant requirements such as eDiary entries

9.8 OUTSIDE OF CCVTM VISITS

Participants will be asked to maintain a diary card describing all (solicited and unsolicited) adverse events up to seven days post vaccination. If there is an ongoing adverse event recorded in the diary the participant will be asked to continue with ediary entries until resolution depending on the nature of the adverse event and feasibility to do so as judged by the clinical investigator. Laboratory results are also entered into a safety results database in real-time or on demand for active monitoring throughout the study by a member of the study team. Each participant will be able to access a member of the study team 24-hours per day via a study-specific emergency number should they have any concerns or are in need of advice.

9.9 LABORATORY INVESTIGATIONS

In addition to blood samples needed for the safe conduct of the trial and assessment of the primary endpoint, blood/oral fluid/stool samples from the participants will also be subjected to laboratory analyses in order to assess the objectives defined in the secondary and exploratory endpoints. The plan for analysis is outlined below, and will be further detailed in a specific analysis plan:

9.9.1 ANALYSIS OF BACTERICIDAL ACTIVITY (GVGH)

The ability of the antibodies in participants serum samples to mediate killing of *S.* Typhimurium and *S.* Enteritidis in the presence of complement (serum bactericidal activity (SBA), will be quantified. The target strains in the SBA assay will be one wild-type strain per serotype in order to elucidate the antigen-specific SBA using a high-throughput luminescence assay developed by GVGH.

9.9.2 FURTHER ANALYSIS OF FUNCTIONAL ANTIBODIES (OVG and Collaborators)

The ability of the antibodies in participants' serum samples to mediate killing of a panel of *Salmonella* bacteria to assess cross protection of the iNTS-GMMA Vaccine. Further exploratory antibody analyses including but not limited to SBAs, opsonophagocytic assay and Fc glycosylation may be performed by OVG and collaborators.

9.9.3 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST O-ANTIGENS (GVGH)

Serum IgG antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. Test samples will be analysed at three dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Anti-OAg responses will be expressed in ELISA units. Plate coating antigens are well characterized OAg purified by GVGH.

9.9.4 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST PORIN AND OTHER ANTIGENS (OVG and Collaborators)

Antibody responses against other antigens from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point may be analysed by ELISA developed and performed by OVG and collaborators. Test samples may be analysed at multiple dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Antigen responses will be expressed in ELISA Units.

9.9.5 CELLULAR RESPONSES AND CYTOKINE RELEASE (OVG and Collaborators)

Laboratory analyses to quantify the B-cell and T-cell responses specific to STmGMMA and SEnGMMA components of the vaccine will be performed when feasible using peripheral blood mononuclear cells (PBMCs) derived from study participants sampled before, and at several time points after each dose, using the assays described below.

9.9.6 ANALYSES OF B CELL RESPONSES (OVG and Collaborators)

The ability of the STmGMMA, SEnGMMA and the iNTS-GMMA vaccine to stimulate a detectable increase in antigen-specific memory B cells and plasma cells will be enumerated by ELISPOT using plates coated with vaccine antigens (such as individual GMMA or their

respective purified O antigen) or B cell mitogens. The phenotype and kinetics of the B-cell subsets involved in the response will be determined using fluorescent-labelled antibodies in a flow cytometric assay. In addition, other assays to monitor the B-cell immune response to the vaccines may be performed if sufficient samples are available.

9.9.7 ANALYSES OF T CELL RESPONSE AND CYTOKINE RELEASE (OVG and Collaborators)

In order to evaluate the ability of the iNTS-GMMA vaccine to stimulate T cell responses, we aim to quantify when possible vaccine-induced responding T-cells by multicolour flow cytometry and mass cytometry. Effector T cells will be clones and rested for antigen specificity against iNTS bacterial strains and tested for their capacity to recognise proteins contained within individual GMMAs. Moreover, other assays to monitor the T-cell immune response and cytokine release to the vaccines will be performed if sufficient samples are available.

9.9.8 ANALYSIS OF GENE EXPRESSION (OVG and Collaborators)

RNA will be extracted from a small volume of peripheral blood (~1ml) at three study visits (as per Table 3.1) for analysis of gene expression profiles. This analysis will be used to highlight differences in gene expression induced by vaccination and provide insight into the immunobiology of vaccine responses.

9.9.9 ANALYSIS OF GENETIC DETERMINANTS OF VACCINE RESPONSE (OVG and Collaborators)

DNA samples obtained, from peripheral blood, will contribute to a Biobank of samples from multiple different Oxford Vaccine Group studies. These DNA samples will be used to analyse the genetic factors influencing vaccine responses (immunogenicity and reactogenicity). DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses. This specific goal will therefore not contribute to the results of this individual study.

9.9.10 ANALYSIS OF ORAL FLUID ANTIBODY CONCENTRATION AGAINST O ANTIGENS AND PORINS (OVG and Collaborators)

Oral fluid samples will be collected via an oral fluid swab as detailed in the Clinical Study Plan. Antibody responses against OAg and porin Ag from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. OAg and Porin Ag responses will be expressed in Antibody Units.

9.9.11 COLLECTION OF SERUM TO BE USED AS A REFERENCE STANDARD FOR THE SET-UP OF LABORATORY ASSAYS IN CURRENT / FUTURE STUDIES (OVG/GVGH and Collaborators)

Serum samples (30mls) will be collected at Visit 8 (28 days post 2nd Vaccine) from recipients of the iNTS-GMMA vaccine within group 3. Serum will be processed as per OVG SOPs and stored at -80°C prior to transfer to GVGH for the development of the serum standard. Serum IgG responses to *S*. Typhimurium and *S*. Enteritidis will be screened by O Antigen ELISA. Samples

will be selected based on ELISA optical density (OD), characterised, calibrated and pooled. The calibrated human serum pool (or serum standard) will allow development of an ELISA standard curve by which test samples may be compared for quantification of antibody titres.

9.9.12 ANALYSIS OF FAECAL ANTIBODY CONCENTRATION AGAINST O ANTIGENS (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing a stool/faeces sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOP. Antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis will be analysed by ELISA. OAg responses will be expressed in Antibody Units.

9.9.13 INVESTIGATION OF IMPACT OF VACCINATION ON GUT MICROBIOTA (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing said sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOPs for bacterial whole genome sequencing and 16S RNA sequencing.

9.10 SAFETY BLOOD TESTS

All other laboratory tests including FBC, WBC differential counts, C-reactive protein, urea, creatinine, electrolytes, aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, will be performed using the OUHFT, NHS laboratories. Blood samples will be collected in assay sample tubes and delivered to OUH clinical laboratories for analysis according to national SOPs.

Samples collected as part of this study may also be used for other exploratory studies of scientific relevance by the OVG laboratory or any of the collaborating laboratories which may include the transfer of samples within and outside the EU. These samples may include oral fluid, serum, extracted DNA and RNA, and PBMCs. Frozen samples will be stored under the ethical approval for this study until study completion. At this time, samples will be transferred to the Oxford Vaccine Centre Biobank subject to participant consent (see Section 9.2). Studies may include further investigation of the inflammatory and immunological response to vaccination.

9.11 TRIAL PROCEDURE SCHEDULE

Details of which assays are performed on each visit are recorded in the table below. The total volume of blood obtained per patient over the course of the study will be 637.5 – 667.5mls.

Visits	Days	Vaccine / Post Vaccine Days	Antibody Assay: SBA, OAg (GSK)	Antibody / Porin Assays (OVG)	PBMCs (T Cell assays)	PBMCs (B Cell assays)	Functional Antibody Assays / Extracted from PBMCs	Serum Standard*	Transcriptomics	FBC	U&E, LFT, CRP	Viral Serology	Oral OAg/Porin IgG/A Assays	Stool/Faecal Ig Assays/Microbiota	Total (mls)
Screen	-	-	-	-	-	-	-	-	-	1	2	4	-	-	7
V1	D0	Vaccine 1	10	5	50	-	+	-	2.5	1	2	-	+	+	70.5
V2	D1	+1	-	-	-	20	-	-	2.5	-	-	-	-	-	22.5
V3	D7	+7	10	-	24	10	-	-	2.5	1	2	-	-	-	49.5
V4	D14	+14	-	5	24		-	-	-	-	-	-	+	-	29
V5	D28	+28	10	5	24	20	+	-	-	1	2	-	+	-	62
V6	D56	Vaccine 2	10	5	24	20	+	-	-	1	2	-	+	-	62
V7	D63	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V8	D84	+28	10	5	24	20	+	30*	-	1	2	-	+	-	62 (92*)
V9	D168	Vaccine 3	10	5	24	20	+	-	-	1	2	-	+	-	62
V10	D175	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V11	D196	+28	10	5	24	10	+	-	-	1	2	-	+	+	58
V12	D350	+182	10	5	24	10	+	-	-	-	-	-	+	+	49
															637.5 (667.5*)

Table 3.1: Blood Sampling (Including Oral Swab) Schedule (in mls)
***Serum standard (30mls) will be collected from participants in Group 3 only**
** Stool Sample collection will be participant opt-in only

Sampling time points, volumes and investigations may vary at the discretion of the PI. Samples may be omitted as per the investigating team's discretion for example if participants develop low haemoglobin as defined and managed as per the SALVO clinical study plan.

9.12 EARLY DISCONTINUATION / WITHDRAWAL OF PARTICIPANTS

Each participant can exercise their right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for participant safety including, though not exclusive to, the following:

- Significant non-compliance with study requirements
- Consent withdrawn
- Lost to follow up

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. The reason for withdrawal, if given, will be recorded in the CRF. A participant who is withdrawn from the study can be replaced if the individual has not received any vaccine. Therefore, lost to follow up subjects who have received at least one dose of vaccine will not be replaced. Furthermore, in circumstances pertaining to the safety of the participant, the

investigator may choose to discontinue further vaccination +/- study procedures, however with ongoing consent may continue to monitor for safety via either scheduled or unscheduled visits. Such circumstances may include though not exclusive to the following:

- Pregnancy
- An adverse event which requires discontinuation of the study vaccinations or results in an inability to continue to comply with study procedures
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. All data and participant samples obtained up to the point of withdrawal will be used in the analysis.

9.13 DEFINITION OF END OF TRIAL

The end-of-study is completion of the last laboratory assay on the last participant sample obtained at Visit 12. End of study must be achieved no later than 8 months after obtaining the last participant last sample at Visit 12.

9.14 SPECIAL CIRCUMSTANCES: COVID-19

9.14.1 STUDY CONDUCT / RISK ASSESSMENT

It is difficult to predict the time course of the COVID-19 pandemic. At all times the safety and welfare of study participants remains paramount.

The Chief Investigator will perform a risk assessment as necessary with relevant parties (e.g. DSMC, Regulatory Authorities or GVGH) on the basis of the current UK COVID-19 situation, to determine:

1. Appropriateness to initiate vaccinations
2. Appropriateness to continue the trial once started
3. Necessity to extend trial duration

Dependant on the prevailing COVID-19 situation, the conduct of the trial may be modified in line with national policy in effect at the time in the interests of participant safety. Such measures may include but are not limited to:

- Pausing further vaccinations
- Modifying study visits and procedures (as detailed in the SALVO Clinical Study Plan) eg: study visits conducted by phone or video calling where appropriate.

Any deviation not outlined in this protocol required due national policy in effect at the time, will require a non-substantial/substantial amendment unless specifically permitted to do otherwise by the Sponsor, MHRA and REC.

9.14.2 COVID-19 INFECTION CONTROL MEASURES AT VISITS

The CCVTM was one of the sentinel sites used by the University of Oxford to implement COVID-19 infection control policies and act as a model for securing a workplace. Further details of infection control procedures including the safe handling of clinic visits, a COVID-19 secure workplace, maintaining staff safety are included in the clinical study plan and OVG SOPs. These will be regularly updated in line with University of Oxford and NHS/UK Government policies.

9.14.3 PARTICIPANTS UNDER QUARANTINE

Given the evolving epidemiological situation both globally and in the UK, should a participant be under quarantine and unable to attend any of the scheduled visits, a telephone/video consultation may be arranged in order to obtain safety and the visit may be re-scheduled, depending on the timelines.

9.14.4 PARTICIPANTS WITH COVID-19 SYMPTOMS

Participants who become symptomatic during follow-up will be instructed to call the study team who will then advise on how to proceed with clinical testing for COVID-19, through the community testing programme, if necessary, as per the Clinical Study Plan. Participants would be expected to report a transient, flu-like illness within 24hours of vaccination. If this reaction should include a fever, we would expect this to resolve within 48hours. If a fever starts and resolves within 48hours of vaccination it will be attributed to the vaccine. If a fever persists for more than 48hours, or starts more than 48hours after vaccination, it will be considered unlikely to be related to vaccination and the participant may be advised to proceed with clinical testing for COVID-19 outside of the study if appropriate.

Participants who develop COVID-19 symptoms and have a positive SARS-CoV-2 test (appropriately validated NAAT or antigen test) after the first vaccination can only receive a subsequent vaccination after a minimum 4 weeks interval from their first positive test of that episode, provided they have had only a mild illness and have fully recovered. Moderate-Severe illness will be defined as 4 or above as per the WHO Clinical Progression Scale (See Appendix D: WHO Clinical Progression Scale for clinical studies of COVID-19). Those who have had moderate-severe disease will not receive further IMP.

In cases of mild or asymptomatic disease, the decision to proceed with booster vaccinations will be at clinical discretion of the investigators, and each case will be evaluated by a study doctor before proceeding (including physical examination and peripheral oxygen saturation recording [Sp02]). The trial clinician must assess that the participant has fully recovered from their illness. Participants must have no ongoing symptoms that could be attributable to their COVID-19 illness and feel that they have fully recovered and are well. For participants who are asymptomatic and have a positive SARS-CoV-2 test (and who remain asymptomatic), a minimum of 4 weeks from positivity will be required before further vaccinations are administered. They will also undergo a physical examination and peripheral oxygen saturation recording before proceeding with IMP.

All relevant details and any required modification in study visits/procedures as per protocol will be documented in a separate adverse event eCRF. Management of these participants will be as detailed above and in the SALVO Clinical Study Plan. Participant follow up and safety reporting will be as detailed in Section 11 Safety Reporting.

9.14.5 PARTICIPANTS INVITED FOR COVID-19 VACCINATION DURING THE TRIAL

Participants who are yet to receive a COVID-19 vaccine but become eligible for this as per UK policy, would be invited to discuss this with the study team.. If agreed by the participant and if it were possible, we would find a mutually agreeable time to receive a COVID-19 vaccine in concert with study timelines and as per exclusion / inclusion criteria (section 8.3). Participants would not be impeded in taking up an offer of a COVID-19 vaccine if offered through the national rollout. If a rollout offer coincides with a planned trial vaccine, the trial vaccine would be rescheduled to 2 weeks before or after the COVID-19 vaccine (whichever was closest to trial schedules). This is in line with the UK Green Book COVID-19 vaccination recommendations for other vaccines which recommends "at least 7 days" interval. It would also minimise the risk of any cross-attribution of reactogenicity, and minimise impact on immunology investigations. If a participant receives a COVID-19 vaccine during the trial, both the vaccine and date of administration will be requested and recorded in the eCRF.

10 TRIAL INTERVENTIONS

10.1 INVESTIGATIONAL MEDICINAL PRODUCT (IMP) DESCRIPTION

10.1.1 iNTS GMMA VACCINE

The vaccine product iNTS-GMMA consists of 2 components: 80 µg OAg/mL STmGMMA and 80 µg OAg/mL SEnGMMA each asceptically formulated independently on Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5. Prior to administration, equal volumes of each component are mixed as specified in the clinical study plan to yield:

- Full dose: 20 µg OAg of STmGMMA/Alhydrogel + 20 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL.
- Lower dose: 5.3 µg OAg of STmGMMA/Alhydrogel + 5.3 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL (3.8 dilution of iNTS-GMMA vaccine full dose diluted with placebo).

10.1.2 PLACEBO

The placebo consists of 0.5 mL Alhydrogel in isotonic 20mM Phosphate buffered saline pH6.5. The placebo is also used for vaccine dilution.

10.2 BLINDING OF IMPS

Group 1-3 will operate a participant-observer blind i.e. both the participant and the observer will be unaware of the group allocation. There will be a trained unblinded team who will prepare and administer the iNTS and placebo vaccine. Both the iNTS and placebo vaccine will appear similar at the point of administration to the participant.

10.3 STORAGE OF IMP

The vaccine product requires storage at 2 to 8oC throughout and vaccines will be transported to the OVG after authorised release for use in the clinical trial by the GSK Qualified Person (QP)

and study approval by EC and MHRA. All movements of study medication between GVGH and OVG, will be documented in accordance with relevant SOPs.

The study treatment will be stored at the OVG in temperature monitored refrigerators with an auditable temperature record in accordance with the manufacturer's instructions and relevant SOPs. Study fridges are connected to a monitoring system with 24-hour access to staff that are able to move the product in the event of significant temperature deviation, for example fridge malfunction as per OVG 001: Vaccine Receipt, Storage, Cold Chain Maintenance and Return/Deposal.

10.4 COMPLIANCE WITH TRIAL TREATMENT

The study investigational product and placebo will be administered by trained unblinded study personnel and will be documented according to GCP guidelines and relevant SOPs. Issues related to compliance are therefore the responsibility of study personnel who have received appropriate training.

Access to the randomisation and vaccination eCRF of the study database will be password protected and restricted to the unblinded study team . In the unlikely event that accidental unblinding (i.e for reasons not outlined in this protocol) of any blinded study team member occurs, this would be recorded as a protocol deviation, and the study team member will be quarantined from taking part in further blinded study activities, in so far as the study progress is not compromised.

10.5 ACCOUNTBILITY OF THE TRIAL TREATMENT

iNTS-GMMA vaccines, placebo (also used as diluent) and empty vials will be manufactured, packaged, labelled and supplied by GVGH. All vaccines (vials and boxes) are labelled with a label specifying 'for clinical trial use only' and no less than the following:

- The clinical trial identifier (by reference code)
- The content of each vial
- Batch and serial number
- Chief Investigator
- Research site

The vaccine will be delivered and stored at the CCVTM pending authorised release for use in the clinical trial.

10.6 CONCOMITANT MEDICATION

The use of all concomitant medication prescribed or over-the-counter, will be recorded in the CRF. There is no restriction on the use of concomitant medication but the use of some prescribed medicines, such as immune suppressive agents, may result in the withdrawal of the participant at the discretion of the Investigator, while others, such as antibiotics, may result in a temporary exclusion.

10.7 EMERGENCY MEDICATION AND PROCEDURES

Participants are required to wait and be observed for one hour after the administration of each vaccine dose for signs of anaphylaxis. All clinical staff are trained and can provide evidence of competency in the acute management of anaphylaxis reactions including the use of intra-muscular adrenaline. This is detailed in relevant SOPs and adrenaline is available at all times of vaccine administration and subsequent observation.

The nearest Accident and Emergency Department is at the Oxford University Hospitals NHS Foundation Trust, which is within minutes by ambulance transfer.

10.8 POST-TRIAL TREATMENT

Study medication will not be continued beyond the trial period.

10.9 OTHER TREATMENTS (NON-IMPS)

No other treatments other than those specified in the protocol above will be administered to trial participants.

10.10 OTHER INTERVENTIONS

No other interventions other than those specified in the protocol above will be administered to trial participants.

11 **SAFETY REPORTING**

11.1 SAFETY REPORTING DEFINITIONS

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

11.2 CAUSALITY ASSESSMENT

The relationship of each adverse event to the trial vaccine(s) or study procedures must be determined by a CI-delegated blinded clinician / investigator. The relationship of the adverse event with the study procedures will be categorized as not related, possibly related, probably related or definitely related. The delegated clinician will use clinical judgement to determine the relationship using the following definitions:

Not related	• No temporal relationship to vaccine administration ***and*** • Alternative aetiology (clinical, environmental or other intervention), ***and*** • Does not follow pattern of recognised response to vaccine administration	
Related	Possible	• Reasonable temporal relationship to vaccine administration, ***or*** • Event not readily explained by alternative aetiology (clinical, environmental or other interventions), ***or***

		• Similar pattern of response to that seen to vaccine administration.
	Probable	• Reasonable temporal relationship to vaccine administration, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***or*** • Known pattern of response with vaccine administration.
	Definite	• Reasonable temporal relationship to vaccine administration or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***and*** • Known pattern of response to vaccine administration.

11.3 SEVERITY ASSESSMENT

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on the criteria listed in the definition of an SAE in section 11.1 above. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Severity will be assessed by clinical symptoms, signs, diagnosis, laboratory results and observations as per the appendices A, B and C. Use of the appendices will be detailed in the following sections.

11.4 PROCEDURES FOR COLLECTING AND RECORDING ADVERSE EVENTS

Abnormal clinical findings from medical history, examination or blood tests, will be assessed by a blinded delegated clinician / investigator as to their clinical significance using the severity grading criteria for Adverse Events tables (see Appendix A, B, C).

All AEs that are observed by the investigator or reported by the participant irrespective of their relatedness to the study medication will be recorded from the day of vaccination and until 28 days after each vaccination These will be recorded in either the e-diary for the first 7 days after each vaccine, the laboratory safety database or the eCRF. Outside of this window (i.e. from 28 days after each vaccination and until the point of a subsequent vaccination or until the final visit if vaccination course completed), non-serious AEs will only be recorded if they require medical attention (contact with GP, visit to emergency department). These will be recorded in the eCRF. All AEs will be collected and recorded by the blinded study team.

It will be left to the blinded investigator clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from further vaccination / study. Such

judgement may require the unblinding of the investigator +/- the participant as per study procedures.

A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. In such an event, Section 9.10 (Early Discontinuation/Withdrawal of Participants) will apply. All AEs that result in a participant's withdrawal from the study will be, subject to participant consent, followed up where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned. This will involve an end of study assessment at which the requirement for further appropriate care under medical supervision will be determined. If required the participant will be referred to their GP for ongoing medical supervision, until symptoms cease or the condition is deemed resolved or stable.

11.4.1 E-DIARY AEs

Solicited adverse events

Solicited AEs are those listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- Solicited adverse events will be recorded by the participant in an electronic diary and graded by the participant alone (appendix A) from the time of each vaccine administration for 7 days post-vaccination (day of vaccination and six subsequent days).

Solicited adverse events will be reviewed daily by the clinical study team. These are participant-entered. If further action is required including face-to-face medical review, and/or prescribed medication this will be recorded by the study team in the eCRF. Causality will be assigned by blinded CI-delegated clinician / investigator. Any solicited AE which meets the definition of a SAE will be managed and reported as per Section 11.6.

Unsolicited adverse events

Unsolicted AEs are those that are NOT listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- These may be recorded by the participant in an electronic diary from the time of each vaccine administration for 7 days post vaccination.

Unsolicited adverse events will be reviewed at clinic visits. If clarification of any event is required then the study nurse or doctor will seek this from the participant during a clinical visit or by telephone call. These unsolicited adverse events will be recorded in the AE section of the eCRF. Unsolicited adverse events recorded in the e-diary will be severity graded by the participant. Causality will also be assigned by the CI-delegated clinician / investigator as per section 11.2.

Additionally participants will be asked about the occurrence of AEs during visits and if any are elicited (within the period for which the eDiary is open) that have not already been recorded they will be recorded in the eDiary as above.

11.4.2 OBSERVATION RELATED AEs

Physical observations (e.g. temperature, blood pressure) of the patient will be taken at each visit (Section 9.6, 9.7). These will be recorded in the eCRF. If abnormal, a severity grading will be automatically assigned by the blinded study team as per Appendix B.

11.4.3 VISIT ELICITED AEs

Participants will be asked about the occurrence of AEs and if any elicited they will be recorded in the eCRF and graded as per Appendix A and B by a blinded CI-delegated clinician / investigator. Any AEs reported outside of the visits will be recorded in the eCRF, except for AEs elicited during the opening period of the eDiary (see Section 11.4.1).

11.4.4 LABORATORY AEs

During the trial, laboratory results will be entered into a password protected database containing all the trial safety blood results. Severity grading for laboratory AEs is defined in Appendix C. Changes in laboratory values will be recorded as AEs if they are of Grade 2 severity or above. Changes of laboratory values of Grade 1 severity may be recorded as AEs if they are judged to be clinically significant by a blinded CI-delegated clinician / investigator.

If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and advised with regards appropriate medical care. Laboratory results may be out of normal range for a number of reasons (eg hot weather, delayed transit to processing laboratory). Changes in laboratory values will be recorded as AEs if they fall out of prespecified ranges and judged to be clinically significant by a blinded CI-delegated clinician / investigator, if some action (eg repeat testing for likely clinically significant test result, or reduction in blood volume required during blood draws) or intervention is required. There may be certain circumstances where it may be necessary to unblind the participant. Should this be necessary the same procedures will be followed as for unblinding participants for SAEs.
If abnormal laboratory values are the result of pathology for which there is an overall diagnosis then this diagnosis should be reported as one AE only.
A Grade 4 laboratory AE will be considered a SAE.

11.4.5 NOTES ON RECORDING AEs

Pre-existing medical conditions (present prior to enrolment into the study) are considered “concurrent medical conditions” and should not be recorded as AEs. However, if the participant experiences a worsening or complication of the condition, the worsening or complication should be recorded as an AE. Study staff will ensure that the AE term recorded captures the change in the condition (e.g., “worsening of”).

Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect at which point it would fall within the definition of "serious" and the congenital abnormality of birth defect would be reported as an SAE. Pregnancy notification and follow-up reports on pregnancy outcome will be provided to the DSMC with ongoing consent of the participant.

11.4.6 FOLLOWING UP OF AEs

AEs considered related to the active vaccine or placebo will be followed until resolution, the event is considered stable or until non-study causality is assigned. At the end of the study all other ongoing/open AEs will be assessed by a blinded CI-delegated clinician / investigator, to ensure if not already done so, adequate medical follow-up (if required) has been arranged, eg. referral to participant's general practitioner.

11.5 REPORTING PROCEDURES FOR SERIOUS ADVERSE EVENTS

SAEs will be collected throughout the entire trial period (from first vaccination to D350 or withdrawal).

All SAEs must be recorded on a SAE form (paper or electronic) with causality assessed by the blinded investigator and reported by email to the CI. All SAE will be reported to the DSMC Chair (or nominated designee) within 24 hours of discovery or notification of the event. If the SAE is deemed related, the CI will unblind to confirm whether the participant has received study vaccine or placebo. If the CI deems that this is a SUSAR, this will be reported according to the SUSAR reporting procedures below. In the absence of the CI these tasks may be performed by a Co-Investigator.

Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form emailed to the CI and DSMC Chair (or nominated designee).

The chair of the DSMC (or nominated designee) will perform an independent review of SAEs and request any further information required in a manner adherent to the procedures and timelines of the DSMC Charter. Documentation of this review will be kept in the TMF. The DSMC will provide independent real-time safety assessment throughout the study as described below.

11.6 EXPECTEDNESS

Expectedness will be determined according to the reference safety information section of the Investigators' Brochure for iNTS-GMMA vaccine. No IMP related SAEs are expected in this study. All SAEs at least possibly related to iNTS-GMMA vaccine will be considered unexpected and be reported to the MHRA and REC as SUSARs within the regulatory timelines, as in section 11.8.

11.7 SUSAR REPORTING

All SUSARs will be reported to the Sponsor, relevant Research Ethics Committee, GVGH, and to the MHRA. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. Any additional relevant information should be sent within eight days of the report.

The CI or Co-Investigator will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

11.8 FORSEEABLE ADVERSE REACTIONS

The foreseeable ARs following vaccination with iNTS-GMMA vaccine or placebo include, locally; injection site pain/ tenderness, redness, swelling, induration; and systemically, headache, malaise, fever, nausea, vomiting, abdominal pain, anorexia, myalgia, arthralgia, and fatigue.

11.9 DEVELOPMENT SAFETY UPDATE REPORTS

In addition to the expedited reporting above, the CI or CI-delegated study team member shall submit once a year throughout the clinical trial or on request, a Development Safety Update Report (DSUR) to the;

- MHRA
- Research Ethics Committee
- Sponsor (RGEA)
- GVGH

11.10 SAFETY PROFILE REVIEW

The safety profile will be reviewed on a day to day basis by the investigators using a blinded electronic diary, adverse events CRF and safety bloods to date. Any concerns will be referred to the blinded CI. If the CI remains concerned they may consider unblinding and/or escalation to the unblinded DSMC as required.

11.11 TRIAL MANAGEMENT GROUP

The OVG study investigators will form the trial management group (TMG) and will provide on-going management of the trial.

11.12 DATA SAFETY MONITORING COMMITTEE (DSMC)

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. Specifically, data review will be done as follows:

1. Formal review of the safety profile after 7 days of safety data has been collected from group 1 before progression to group 2, and review of group 2 after 7 days of safety data has been collected before progression to group 3, as described in section 7.
2. Formal review of the safety profile after two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the

second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

3. Independent review following any SAE deemed to be related to the trial active vaccine or placebo.
4. Unscheduled reviews on request of the study management committee at a demand and frequency determined by the severity of reported adverse events.

From these reviews the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. A summary of all blinded and unblinded AEs and SAEs to date will be provided to the DSMC on request.

The outcome of each DSMC review will be communicated directly to the TMG and documentation of all reviews will be kept in the TMF. The CI will inform GVGH of the outcome of the DSMC review.

The Chair of the DSMC will also be contacted for advice where the Chief Investigator feels independent advice or review is required.

11.13 OTHER SAFETY REVIEWS

In addition to formal DSMC review, there will be local blinded safety monitoring reviews. As described in section 7.2 and 7.5, there will be formal local blinded reviews of safety data by the CI or CI-delegated clinician / investigator at:

1. Day 2 following vaccination of the first paired cohort in groups 1 and 2, to decide on progression to vaccination of respective second and third paired cohort.
2. Following 7 days of safety data after the last participant to receive their second vaccine in group 1, to decide on progression to administer second vaccines in group 2.
3. Following 7 days of safety data after the last participant to receive their second vaccine in group 2, to decide on progression to administer second vaccines in group 3.
4. Following 7 days of safety data after the last participant to receive their third vaccine in group 1, to decide on progression to administer third vaccines in group 2.
5. Following 7 days of safety data after the last participant to receive their third vaccine in group 2, to decide on progression to administer third vaccine in group 3.

11.14 GROUP HOLDING RULES

Group holding rules are as follows:

SAE

- All Grade 3 adverse events in any individual which is possibly, probably or definitely related to vaccination (i.e. a AR) will be assessed by the CI-designated clinician / investigator to determine whether the event meets the criteria for a SAE related to vaccination (i.e. a SAR) as per Section 11.1. If this criteria is met the this would trigger the following group holding rule.
- An SAE which occurs in any one individual which is possibly, probably or definitely related to vaccination (i.e. a SAR) would trigger a group holding rule.

Solicited/unsolicited/laboratory adverse events

Solicited local adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 solicited local adverse event within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs

Solicited systemic adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any groups are followed by a Grade 3 solicited systemic adverse event beginning within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs.

Unsolicited adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 unsolicited adverse event within 7 days after vaccination (day of vaccination and six subsequent day) and persisting at Grade 3 for >48 hrs.

Laboratory adverse event:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 laboratory adverse event beginning within 3 days after vaccination and not significantly improving (on clinical judgement), persistent or worsening on repeat testing at a clinically appropriate interval.

If the holding rule has been met and following a safety review by the DSMC it is deemed appropriate to restart dosing or to continue only with the lower dose of vaccine, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The DSMC safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Study Information Booklet (SIB) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The local ethics committee, MHRA, GVGH and the Sponsor will be notified if a holding rule is activated or released.

All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

11.15 INDIVIDUAL HOLDING RULES

In addition to the above stated group holding rule, stopping rules for individual volunteers will apply (i.e. indications to withdraw individuals from further vaccinations):

- **Local reactions:**
  - Injection site ulceration, abscess or necrosis

- **Laboratory AEs:**
  - the volunteer develops a Grade 3 laboratory adverse event considered related within 7 days after vaccination, not significantly improving (on clinical judgement), persistent or worsening on repeat testing.

- **Solicited adverse events:**
  - the volunteer develops a Grade 3 systemic solicited adverse event considered related within 7 days after vaccination (day of vaccination and six subsequent days), persisting continuously at Grade 3 for > 48hrs.

- **Unsolicited adverse events:**
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, persisting continuously at Grade 3 for > 48hrs,
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, which is considered a serious adverse event as assessed by the CI-designated clinician / investigator,
  - the volunteer has a serious adverse event considered related to vaccination, or
  - the volunteer has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.

If a volunteer fulfils any of the temporary exclusion criteria (see section 7) at the scheduled time of a second administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol (see Table 1) or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated volunteers will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

11.16 STOPPING RULES

The trial will be discontinued in the event of any of the following:

- New scientific information is published to indicate that subjects in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the subjects enrolled in this or any other on-going trial of the GMMA vaccine delivery system.
- For any other reason at the discretion of the Chief Investigator or DSMC.

Additionally, the DSMC can temporarily pause the trial if time is required to reach a decision regarding stopping the trial e.g. to determine causality for SAE.

12 STATISTICS

12.1 DESCRIPTIVE STATISTICAL METHODS

The analyses for this study will be descriptive in purpose and will not include any hypothesis testing or presentation of p values for group comparisons or power calculation.

12.2 THE NUMBER OF PARTICIPANTS

30-42 participants will be recruited to the study allocated to groups 1-3 as detailed in section 7. Participants will be replaced only if they have not received a dose of vaccine. There has been no formal power calculation to determine this figure as the study is primarily descriptive. The number of participants has therefore been chosen to pragmatically reflect logistical and budgetary constraints.

12.3 THE LEVEL OF STATISTICAL SIGNIFICANCE

There will be no statistical significance testing. All confidence intervals for descriptive analyses will be set at 95%.

12.4 CRITERIA FOR TERMINATION OF TRIAL

The Chief Investigator and Data Safety Monitoring Committee will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the investigator will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the MHRA, GVGH and relevant Ethics Committee will be notified within 15 days of this occurring.

In the event of the trial being terminated early, follow-up of enrolled participants will still continue as detailed in tables 2.1 for safety reasons, with the exception that further vaccination will not be given and study procedures will be modified to monitor safety only.

12.5 PROCEDURE FOR ACCOUNTING FOR MISSING, UNUSED, AND SPURIOUS

All available data will be used in the analyses and there will be no imputations for missing data. Participants will be analysed according to the group to which they were assigned.

12.6 INCLUSION IN ANALYSIS

All participants with any available data will be included in the analyses.

12.7 INTERIM ANALYSIS

An interim analysis of the secondary objective, immunogenicity at two dose levels (lower dose and full dose iNTS-GMMA Vaccine) will be performed once enrolement is complete and once all second vaccine + Day 28 (Day 84; V8) samples have been collected from all groups. An interim analysis of some of the exploratory objectives may also be performed dependant on validation of the exploratory assays.

13 DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

The investigators will populate the content of participants' CRFs, which will be in a paper and/or electronic format using an REDCap database (or an appropriate alternative). This database is stored on a secure University of Oxford server and has restricted access and is password-protected with accountability records. This data includes safety data, laboratory data (both clinical and immunological) and outcome data. All information transcribed to and from the REDCap database is by encrypted (Https) transfer.

Each study participant will have a unique screening number which will be allocated following the taking of informed consent and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number which will be determined by enrolment order and initials as a second identifier. Samples sent to laboratories for processing will be identified by a trial number and participant number only.

13.1 DATA INTEGRITY

Data collection and storage will be inspected throughout the study by internal (performed by the Oxford Vaccine Group) and external (Appledown Clinical Research Ltd) monitoring. The Sponsor may also audit the trial data.

13.2 DATA ARCHIVING AND STORAGE

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked filing cabinet at the site. All essential documents will be retained for a minimum of 5 years after the study has finished. Volunteers who complete online screening or telephone screening only (before informed consent) will not have data kept beyond the end of the trial. The need to store study data for longer in relation to licensing of the vaccine will be subject to ongoing review. For effective vaccines that may be licensed, we may store research data securely at the site at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for 7 years in line with the site financial policy. De-identified research data maybe be stored indefinitely.

13.3 SOURCE DATA

Source documents are original documents, data, and records from which participants' CRF data is populated. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study CRF entries will be considered source data where it is the site of the original recording. All documents will be stored safely under strict confidentiality and with restricted access. On all study-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the study participant number/code only.

13.4 ACCESS TO DATA

Direct access will be granted to authorised representatives from the sponsor/host institution (including Appledown clinical Research Ltd), GSK/GVGH and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.5 DATA RECORDING AND RECORD KEEPING

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. REDCap, or similar) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment. REDCap is widely-used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored in a separate password protected Access databased saved on a secure University of Oxford server. Only Oxford staff have access to the Access database and are permitted for data entry.

Each study participant will have a unique participant number which will be allocated at the time a screening visit is booked and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number and/or code. Samples sent to laboratories for processing will be identified by trial number and participant number only.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g. requesting to be informed of other trials), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Bank details will be stored for 7 years in line with University financial policy.

14 QUALITY ASSURANCE PROCEDURES

14.1 RISK ASSESSMENT

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

14.2 MONITORING

Regular monitoring will be performed by Appledown Clinical Research Ltd according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

15 PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file as per SOP.

16 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.
A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority, the relevant NHS host organisation and GSK/GVGH within seven calendar days.

17 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 DECLARATION OF HELSINKI

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

17.2 GUIDELINES FOR GOOD CLINICAL PRACTICE

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

17.3 APPROVALS

Following sponsor approval the protocol, informed consent form, participant information sheet and required material will be submitted to an appropriate Research Ethics Committee (REC), MHRA, regulatory authorities, and host institution(s) for written approval.
The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4 TRANSPARENCY IN RESEARCH

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17.5 REPORTING

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and summary report will be submitted to the MHRA, the REC, host organisation and Sponsor.

17.6 PARTICIPANT CONFIDENTIALITY

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number and initials on all trial documents and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

17.7 PARTICIPANT REIMBURSEMENT

Each participant is compensated for their time and for the inconvenience based on the following figures:

- Travel expenses: £15 per visit
- Inconvenience of blood tests: £10 per visit
- Time required for visits: £20 per visit

Remuneration is on a *pro rata* basis should a participant fail to complete all visits and/or study requirements. Each participant can therefore receive a maximum of £585. Payments will be made in instalments after V0, V6, V10, and V12.
Additional reimbursement for unscheduled visits at £45 per visit will be provided. This will not be given unless an unscheduled visit occurs.

18 FINANCE AND INSURANCE

FUNDING

This clinical trial is funded by a European Union Horizon2020 grant. Additional budget from GVGH will cover necessary costs for monitoring activities not already funded by the Vacc-iNTS European Union Horizon2020 grant.

18.2 INSURANCE

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London

18.3 CONTRACTUAL ARRANGEMENTS

Appropriate contractual arrangements will be put in place with all third parties.

19 PUBLICATION POLICY

The Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator prior to submission.

20 DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP derived from this trial will be in accordance with the Consortium Agreement signed by Beneficiaries of the Horizon2020 Vacc-iNTS EU Grant No 815437.

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APPENDIX A: GRADING THE SEVERITY OF SOLICITED AND UNSOLICITED ADVERSE EVENTS

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6
	1	37.6 – 38.0
	2	38.1 – 39.0
	3	> 39

Adverse event	Grade	Definition
Any symptom	0	Absence or resolution of symptom
	1	Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3	Significant interference with daily activity
	4	Emergency department visit or hospitalisation
	5*	Fatality

*All grade 5 AE will be considered either a SAE, SAR, or SUSAR dependant on causality and 'expectedness'

APPENDIX B: GRADING THE SEVERITY OF VISIT OBSERVED ADVERSE EVENTS

Observation	Grade 1	Grade 2	Grade 3
Oral temperature (ºC)	37.6 – 38.0	38.1 – 39.0	>39
Tachycardia (beats/min)	101-115	116-130	>130
Bradycardia (beats/min)	50-54	45-49	<45
Systolic hyper-tension (mmHg)	141-150	151-155	>155
Diastolic hyper-tension (mmHg)	91-95	96-100	>100
Systolic hypo-tension (mmHg)	85-89	80-84	<80

The following ranges are considered normal physiological ranges and are recorded as Grade 0:

- Oral temperature between 35.5 and 37.5 C
- Resting heart rate between 55 and 100 beats/minute
- Systolic blood pressure between 90 and 140 mmHg

APPENDIX C: GRADING THE SEVERITY OF LABORATORY OBSERVED ADVERSE EVENTS

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
Haemoglobin: decrease from baseline value (g/l)	≤ 15	16-20	21-50	>50
White cell count: elevated (10^9/L)	11.5–15	>15–20	>20–25	>25
White cell count: depressed (10^9/L)	2.5-3.5	1.5-2.49	1.0-1.49	<1.0
Neutrophil count (10^9/L)	1.5-1.99	1.0-1.49	0.5-0.99	<0.50
Platelets (10^9/L)	125-140	100-124	25-99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150
Potassium: hyperkalaemia (mmol/L)	5.1–5.2	5.3–5.4	5.5–5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.3–3.4	3.1–3.2	3.0	<3.0
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	114-156	157-312	>312	RRT
ALT and/or AST (IU/L)	1.1–2.5 x ULN	>2.5–5.0 x ULN	>5.0-10 x ULN	>10 x ULN
Bilirubin, with increase in LFTs (µmol/L)	1.1–1.25 x ULN	>1.25–1.5 x ULN	>1.5–1.75 x ULN	>1.75 x ULN
Bilirubin, with normal LFTs (µmol/L)	1.1–1.5 x ULN	>1.5–2.0 x ULN	>2.0–3.0 x ULN	>3.0 x ULN
Alkaline phosphatase (IU/L)	1.1–2.0 x ULN	>2.0–3.0 x ULN	>3.0–10 x ULN	>10 x ULN
Albumin: hypoalbuminaemia (g/L)	28–31	25–27	<25	Not applicable
C-reactive protein	>10-30	31-100	101-200	>200

Grade 4* Potentially life threatening

APPENDIX D: WHO CLINICAL PROGRESSION SCALE FOR CLINICAL STUDIES OF COVID-19

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

APPENDIX E: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3	14/06/2022	Brama Hanumunthadu/Nelly Owino/Maheshi Ramasamy	• Edit to Typhoid / Paratyphoid vaccine as an exclusion criteria • Clarification of SARS-COV-2 test as a temporary exclusion criteria • Addition / modification of mailout language and inclusion of use of GP databases to identify potential participants
NSA01	3.1	13/01/2023	Timothy Crocker-Buque	• The protocol has been has been edited to clarify: • Minor edit to Participant study windows for the V5, V8 and V11 (D28 post vaccination Visit). Edit changed from D28 +/- 4 days to +/- 7 days. This change has been made to improve participant management and Data collection.

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC / MHRA / HRA submission.
Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, HRA (where required) or MHRA.

Oxford Vaccine Group
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Sal*monella* **V**accine Study in **O**xford

SALVO

INFORMED CONSENT FORM

Participant's Name: _____

Participant Initials: _____

Participant Number: S A L ___ ___ ___

If you agree, please initial box:

Section 1: Study Procedures	
1. I confirm that I have read the information sheet dated..... (version.....) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I have spoken with Dr/Nurse_____	
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
4. I have received detailed information about the intervention schedule, study procedures, potential side effects and their importance.	
5. I agree to be randomised to receive either the iNTS-GMMA vaccine (Lower or Full Dose Schedule) or the placebo schedule as detailed in the participant information sheet and I am aware of the risks and side effects associated with each intervention. I am aware that each schedule includes 3 vaccine or placebo administrations.	

6. I will bring the 24-hour contact reply slip, signed by my 24-hour contact prior to receiving the first dose of vaccine or placebo. I agree that the study team may contact this person if I cannot be contacted during the study.	
7. I agree to refrain from donating blood/blood products for the duration of the study.	
8. ***Women only***: I understand the need to ensure that I or my partner use effective contraception one month prior to first vaccination and continue to do so for the remainder of the study.	
Section 2: Personal Information	
9. I agree to OVG storing and using my personal information as described in the information booklet.	
10. I agree to my General Practitioner being informed of my participation in this study. I agree to my GP and/or other treating doctors being approached for additional information regarding my medical and vaccination history and study staff to access my NHS medical records either via my GP or the electronic patient records system.	
11. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University of Oxford (Sponsor), from regulatory authorities [and from the NHS Trust(s)], where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I agree to my National Insurance (if UK citizen) or Passport number being used to register me on TOPS. I understand that it will be stored electronically for the duration of the study.	
12. I understand TOPS is a Health Research Authority database that aims to prevent healthy volunteers from taking part in too many studies. I understand that only staff at OVG and other research units can use the database and OVG may call other units, or OVG may be called, to check volunteer details.	
13. I agree to provide my bank account details including my account name, sort code and account number for reimbursement purposes. I understand that my banking details will be stored electronically as described in the information booklet. I understand that my personal information will be shared to the extent required to process or verify eligibility of payments as described in the information booklet.	
Section 3: Research Samples and Data	
14. I agree to donate blood and saliva samples. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	

15. I agree to my de-identified data and biological samples being sent and stored within and outside of the European Union for analysis by collaborating research groups as described in the information booklet.		
16. I understand and agree that some of my samples will be used to investigate the genetic factors determining the response to iNTS-GMMA vaccine or placebo.		
If all of the applicable sentences above are initialled, meaning "yes", then please continue:		
17. I agree to take part in this study.		
Optional:		
18. I agree to donate stool samples for this study. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	Yes	No
19. I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacted does not oblige me to participate in any further studies.	Yes	No

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 original for researcher site file.*

OXFORD VACCINE GROUP

*Sal*monella Vaccine Study in Oxford
SALVO

PARTICIPANT INFORMATION SHEET

You are invited to take part in a study to test a new vaccine against Invasive Non-Typhoidal *Salmonella* (iNTS), an important cause of blood poisoning in children and adults in sub-Saharan Africa. The study is being run by the Oxford Vaccine Group, which is part of the University of Oxford.

Participation in the study is entirely voluntary. Before you decide whether to take part, it is important for you to understand what the study is about and what participation would involve. Please take time to read the information carefully and discuss with others if you wish. If anything is unclear or you would like further information, please contact the study team.

Thank you for taking the time to consider taking part in this study.

Contact Details
Oxford Vaccine Group
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital
Oxford OX3 7LE
Tel: 01865 611400
Email: info@ovg.ox.ac.uk
Website: www.ovg.ox.ac.uk

Who are the Oxford Vaccine Group?

The Oxford Vaccine Group, which is part of the **University of Oxford**, is an independent research team of doctors, nurses and play assistants. We carry out research studies of new and improved vaccines for babies, young children, teenagers and adults, and teach doctors and nurses about immunisations. In the past 5 years alone, over 7,000 participants in the Thames Valley area have taken part in our research studies.

What is invasive Non-Typhoidal *Salmonella* disease?

Non-typhoidal *Salmonella*e are a group of bacteria, that are well known to cause food poisoning throughout the world. However, in certain circumstances they can cause a more serious disease, where *Salmonella* can spread beyond the gut leading to blood poisoning, and in some cases sepsis and death. This is called invasive non-typhoidal *Salmonella* (iNTS) disease and is of particular concern in individuals with a weaker immune system. iNTS disease occurs in over half a million people a year, and particularly affects children under 5 years of age in sub-Saharan Africa, where it causes significant disease with over 200,000 cases and 31,000 deaths per year. Adults can also be affected by iNTS disease, particularly those with HIV, malaria or malnutrition.

What is the purpose of the study?

In this study we are investigating a new vaccine against iNTS called the iNTS-GMMA vaccine. This new vaccine is developed by the GSK Vaccine Institute for Global Health (GVGH), a GlaxoSmithKline (GSK) company based in Italy with which the Oxford Vaccine Group is collaborating for the development of a vaccine against iNTS. This will be the first time this vaccine will be given to human volunteers. It contains small amounts / particles of the outer surface of the two most common bacteria that cause iNTS disease (*Salmonella* Enteritidis and *Salmonella* Typhimurium). These particles previously known as Generalised Modules for Membrane and Antigens', and currently abbreviated to 'GMMAs', constitute the main component of the vaccine. **The vaccine does not contain *Salmonella* bacteria and therefore cannot cause infection or disease**. It is hoped that these GMMA particles can stimulate the immune system to produce a protective response against iNTS bacteria and thus prevent future blood stream infections by these bacteria. The GMMA particles are diluted in Alhydrogel, a common vaccine component designed to reduce local side effects. The study is being conducted to evaluate the safety of the vaccine and how well it stimulates the immune system against iNTS.

Not everyone will receive the active vaccine, some individuals will receive a placebo (non-active comparison). The placebo contains all the components of the iNTS-GMMA vaccine except for the active GMMA particles i.e. Alhydrogel alone and cannot stimulate the immune system to produce a protective response against iNTS bacteria. Participants will be enrolled sequentially into 3 groups and randomly allocated to receive either the iNTS-GMMA vaccine or the placebo. Randomization means that neither you nor your doctor will choose whether you receive the active vaccine or placebo. In this study, a computer will assign this to individual participants, like flipping a coin. You have an equal chance of receiving the vaccine versus placebo in groups 1 and 2 and have more chances of receiving the vaccine than the placebo in group 3.

Why have I been invited?

We are inviting healthy adults aged 18 to 55 years old to take part in this study. We would not want to recruit anyone who has significant health issues, anyone with altered immune function or any females who are pregnant or thinking of starting a family soon. We use various ways to contact potential volunteers, including the Electoral Roll and the National Health Applications and Infrastructure Services (NHAIS) who hold the central NHS patient database (Open Exeter). This database identifies all persons within the local area who are in the appropriate age range. Whilst we do commission the invitation to take part in the study, we do not have access to personal data and are not directly responsible for the mail out. In addition, you may have previously expressed an interest in taking part in studies at the Oxford Vaccine Group.

What should I consider?

We are keen to recruit healthy volunteers who are:

- Willing to take part and able to attend all study visits
- Aged between 18 and 55 years old, inclusive
- Willing to allow us to communicate with their GP to notify them of your participation in the study and to check your medical history
- (Females) Willing to use effective contraception from 1 month prior to vaccination and for the remainder of the study

Do I have to take part?

No. We are looking for volunteers. Should you volunteer and later change your mind (for whatever reason) it is your right to do so, and you would not need to provide an explanation to the study team or anyone else. In addition, your decision to withdraw would not affect any ongoing medical care you are or will be receiving.

Whatever you choose it's important that you are happy with your decision and it is not the role of the study team to decide for you. If you choose to withdraw after the receiving the vaccine, we would stop all research related activities. However, we would like to check that you remain well after receiving the vaccine for your own safety which may or may not include follow up visits / blood tests. We would use the samples and data we have collected from you in our analysis of the study, up until the point you informed us that you wanted to withdraw.

What are the possible benefits of taking part?

There are no clear benefits to you if you take part in this study. However, you would have the knowledge that you played a part in the early stages of developing a new vaccine against a bacteria that causes a significant burden of death and disease, particularly in sub-Saharan Africa and in children under 5 years of age for which there are currently no licensed vaccine.

Are there any possible disadvantages or risks from taking part?

In general, the risks are in relation to the vaccine/placebo, blood and oral fluid sampling. In addition, you would be asked to attend regular visits at the CCVTM. In regards to COVID-19 please see the COVID-19 section below.

- **Vaccine**

General Vaccines

Intra-muscular vaccination can commonly cause reactions, although most tend to be minor and only last a few days. These may typically include injection site - discomfort, redness, and swelling. As for all vaccines some volunteers occasionally may feel generally unwell, develop fevers, muscle aches, joint aches, headache, experience loss of appetite, nausea / vomiting, abdominal pain or diarrhoea. Not everyone will experience symptoms and if they do occur, they should resolve after a few days.

Anaphylaxis is a very rare but a potentially life-threatening allergic reaction and may occur after immunisation. All clinical staff are trained in the immediate treatment of anaphylactic reactions including the use of intra-muscular adrenaline. It is for this reason you need to wait at least 60 minutes after each vaccine dose is given, as this would be within the typical time frame should this reaction occur.

iNTS GMMA Vaccine

This study is the first time that the iNTS-GMMA vaccine will be given to human participants. The studies performed in animals prior to moving on to human trials have shown good safety results. In addition, GMMA-based vaccines against other bacteria have been safely used in over 190 volunteers. These vaccines were found to be well-tolerated and safe in the volunteers who received them. Nevertheless, this is a new vaccine and there may be side effects we do not know about. It is important for you to be aware of this. However, we have multiple measures in place to ensure your safety during the trial as outlined below.

Throughout the study, the safety of the participants in all groups will be monitored following vaccination. This will be done by reviewing of symptoms at visits and through the electronic Diary (eDiary). For your safety there will be an independent safety committee consisting of an independent panel of experts known as the Data and Safety Monitoring Committee (DSMC) who will know whether you have received vaccine or placebo in order to periodically monitor the overall safety of the trial. This committee will review the safety data particularly as the trial proceeds between Groups 1, 2 and 3 and would be required to approve progression from lower to full dose active iNTS-GMMA vaccine within the trial. Should there be any concerns this committee has the power to halt the trial for further evaluation. In addition, you will be provided with study team contact details who are available 24/7 should you need to contact us.

We would ask you to provide contact details of a person who would act as a second contact. Only to be used in an emergency or needing to contact you urgently.

- **Placebo**

The placebo (Alhydrogel) is a common component used in many vaccines and would not usually cause any side effects other than the ones known for any general vaccine as listed

above. Not everyone will experience side effects and if they do occur, they should resolve after a few days.

- **Blood/Oral Fluid Sampling**

Blood tests can be painful and sometimes leave bruising and/or temporary discomfort, but these all resolve in a very short period of time. Rarely fainting can occur. Oral fluid samples are collected with a cotton swab of the mouth and should not cause any discomfort.

- **Pregnancy**

For females, you should not take part in this trial if you are pregnant or breastfeeding. It is currently unknown whether the vaccine being tested is safe during pregnancy. For this reason, it is important that women use adequate contraception during the study period. Women who are not of childbearing potential (i.e. postmenopausal or permanently sterile due to surgery such as a hysterectomy) will not be required to use contraception. This will be discussed with you at the screening visit. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the trial, although we will ask to follow you up for safety reasons.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible.

- **COVID-19**

It is difficult to predict the time course of the COVID-19 pandemic. Should further peaks occur during the study, we will implement specific measures to ensure your safety whilst taking part in the study. **The safety of our study participants remains the prime consideration during the trial.** If you have not already received a COVID-19 vaccine and you become eligible (according to UK policy) for the vaccine during the study, we would talk to you about this. If you agreed and if it was possible, we would find a mutually agreeable time for you to receive this vaccination. We would not prevent you from receiving this vaccination. During the study we will follow up-to-date government advice including on advising testing, self-isolation, and personal protective equipment (PPE) as necessary. Should you develop symptoms of COVID-19 or test positive for COVID-19 during the study, we would want to know about this prior to coming to clinic. This may affect the timings of your clinic visits and we would want to assess whether it is safe for you to receive further study vaccinations.

What will happen to me if I decide to take part?

- **Recruitment**

If you express an interest in taking part, a member of the Oxford Vaccine Group will contact you by telephone to discuss the study and answer any questions you may have. If you have accessed the participant information sheet via the online questionnaire you will have already answered initial eligibility questions. We would like to ask you a few more detailed questions to assess your eligibility.

Following this, if you are interested and seem suitable for the study then we would arrange for you to come to our clinic for a screening visit. In addition we would send you a consent

form (paper or electronic) giving permission for the study team to access your medical records to obtain this information via the electronic patient records or through your GP. We would then ask you to return a copy of the signed consent form (paper or electronic). A countersigned form will be provided at the screening visit. This consent form is only to allow access to your medical records, and not the consent for enrolment on to the study. If you choose to participate in the study a separate consent will be taken for inclusion into the trial.

- **Screening Visit**

The **purpose of screening tests is to ensure that you are eligible to take part** and that by taking part in the study you are not taking on any extra risk to your health. At the screening visit we would sit with you and go through the study in detail. This visit would provide an opportunity for you to ask any questions you might have about the study and what's involved. You would be allowed as much time as you feel necessary before making any decision on whether to take part.

Is coming to screening a commitment to taking part?

No. It's an opportunity to meet with the study staff and ask questions; you do not need to make a decision there and then.

What if I wish to volunteer?

If you are keen to proceed, we would ask you to sign an **informed consent form**. Only once this is signed would we then start any study procedures.

What are the study procedures at the screening visit, if I decide to volunteer?

We would ask you questions about your health, undertake a physical examination and take a blood sample (of 10ml; approximately two tablespoons). We would also require a urine sample. Blood testing includes HIV and hepatitis B and C tests, as well as screening for anaemia, liver and kidney function. For females, we would perform a pregnancy test on your urine sample.

Demographic data including your name, date of birth, gender, ethnicity and contact details will be collected, if not already recorded at the recruitment stage. Data protection regulation governs how we store and process your data. Please see 'What will happen to my data?' below for further information.

We would also seek your consent to register your name on the 'The Over-volunteering Prevention System' (TOPS) national database. This is designed to guard against the potential for harm that can result from excessive volunteering in clinical trials involving investigational medicinal products and blood donations. This would be done using your National Insurance number or passport number, and all information is kept confidential. More information can be found at http://www.hra.nhs.uk/about-the-hra/our-committees/the-over-volunteering-prevention-system/. If we have not already done so, prior to your screening visit we would also ask for your consent to access your medical records via the electronic patient record system or via your GP to obtain any relevant medical history that may affect your participation in the study. Once the study team have confirmed your suitability for the trial, we would inform you and arrange a date for your

first visit. You would be formally enrolled into the study at the time the first vaccine dose is given.

- **Enrolment**

If you are eligible for the study, you will be enrolled into 1 of 3 groups (outlined below) dependant on your enrolment date:

Group 1	Randomly allocated 1:1 to receive either the lower dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 2	Randomly allocated 1:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 3	Randomly allocated 2:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to eighteen participants will be allocated to this group.

Table 1: Enrolment of Groups 1-3

- **Overview of Study Visits**

Each participant would receive 3 vaccinations with the iNTS-GMMA vaccine or placebo at intervals of 0, 2 and 6 months. In addition, each participant would require blood tests immediately before each vaccination and at specific intervals after each vaccination. The study will require a total of 12 visits over a 12-month period.

A simplified overview of the study is shown in the diagram below:

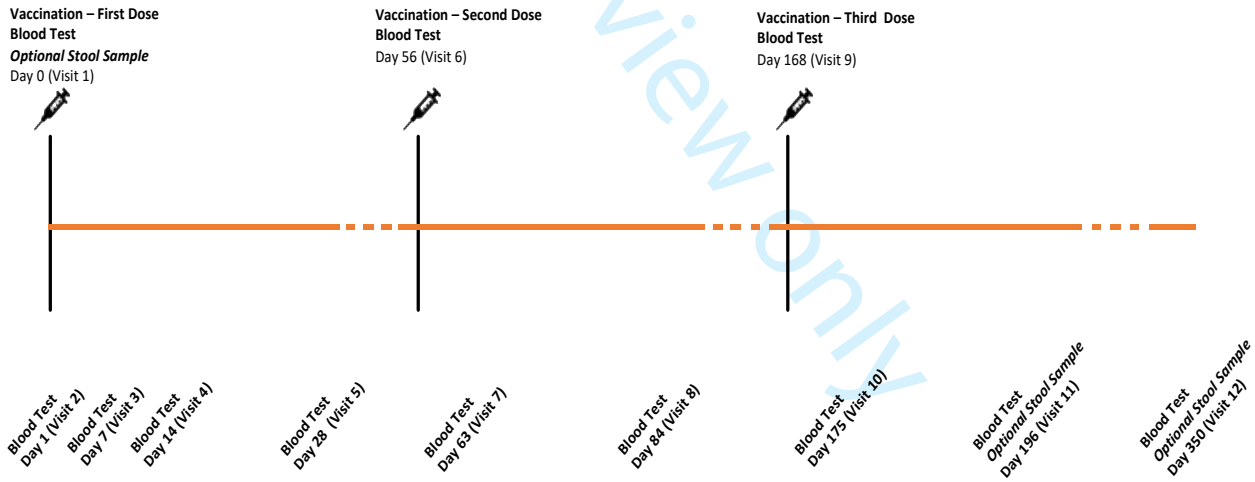

Figure 1: Overview of Study Design

Neither you nor the study team analysing the data will know whether you have received the iNTS-GMMA vaccine or the placebo. This is termed a 'participant-observer blind' trial and provides greater confidence in the final conclusion of the study.

*For participants in group 3 we will collect an additional blood sample (30mls, equivalent to 6 tablespoons) to develop a serum standard at Visit 8 (Day 84). The purpose of the serum

standard is to create a supply of antibodies directed towards the iNTS-GMMA Vaccine that can be used as a reference standard to develop laboratory tests and compare how well this vaccine or future vaccines are working in the individuals being vaccinated. Due to the study design participants who receive the placebo will also donate this additional sample. While these samples will undergo the same laboratory tests, these samples will not be used as a reference standard but may be used in laboratory test development.

- **Vaccine Visits (Visit 1, 6 and 9)**

You would be given a date, time and place to come to the Oxford Vaccine Group at the Churchill Hospital. We would start by checking that you are happy to remain in the study and ask if anything has changed medically since we last saw you.

We would check your pulse and blood pressure at every visit, and for all women a pregnancy test would be done prior to administering each dose of vaccine or placebo. We would take blood and oral samples and give the first vaccine by injection into the muscle of the upper arm.

After vaccination you would need to wait with us for 60 minutes before leaving. This is standard practice to monitor any reactions to the vaccine.

We would give you a tape measure, thermometer and set you up on the **electronic diary** for you to record any symptoms or side effects and daily temperatures for the next **7 days following vaccination**. We would explain how you record this information when you are with us, and it should be entered electronically via a secure link and encrypted transfer on our website wherever possible. Monitoring the ediary will allow the team of research doctors and nurses to check on your wellbeing and ensure your safety.

- **Follow-up Visits**

There are two to four scheduled follow up visits following each vaccination. These visits usually last approximately 30 minutes. We would check on your progress during the trial, review any ediary entries, take recordings of your pulse and blood pressure and collect any blood or oral fluid samples as per the sample schedule below.

- **Samples**

We collect a blood sample at each visit. The amount will be between 5mL (about 1 tablespoon) and 70.5 mL (quarter of a cup) depending on the tests that will be done. The total amount of blood taken during the whole study (1 year) would be up to 667.5mL (about 3 cups), which is significantly less than the maximum amount of blood that could be donated over a year to the UK Blood Donation Service. This is in line with the blood donation guidelines and your body would replace this naturally after about three months. Repeated blood tests can cause anaemia (low haemoglobin), however we have checks and processes in place to ensure this is minimised, including reducing the blood volume we take from you. In rare cases we may ask you to see your general practitioner for a medical review. These blood tests are important to measure how good the vaccine is at producing an immunity to iNTS. In addition, we periodically check your general blood tests including full blood count, urea and electrolytes and liver function tests to ensure they are normal throughout the study.

At some visits we will collect an oral fluid sample using a simple 10 swab (1-2 minutes). In addition, to blood we are testing oral fluid to measure indicators of immunity to iNTS.

In addition, stool samples may be collected during the study. This is entirely **optional**. If you do not agree to the collection of stool samples, this will not affect your participation in the study. If you opt-in, the stool samples will be collected before the first vaccine is administered (Visit 1), 28 days after the third vaccine (Visit 11) and at the end of the study (Visit 12). We will test for the microscopic organisms such as bacteria, parasites and fungi that naturally occupy your gut to see if the mix of these microscopic organisms influences your response to the study vaccine. In addition we will test for the development of gut antibodies to Non-typhoidal *Salmonella*e. You will be given collection materials and the study team will explain how to collect the samples. We will receive the samples at the specified visits. However, if you are unable to provide a sample there is an option to free post if you are happy to do so. You will be given 'By Post' collection materials and the study team will explain how to collect and post the sample.

There might be times when your blood needs to be retested or a urine sample taken to confirm test results. This might happen at your next visit or at an unscheduled visit that you would need to come to the clinic for. At this visit the study staff would take a sample of your blood (usually 10 mL, 2 tablespoons) and collect a urine sample, as needed.

What will happen to the samples I give?

Your sample will be assigned a code and your data will also be identified only by this number. The material given to researchers will not have information that directly identifies you. The blood, urine, stool and oral fluid samples collected during this study would be analysed in the Oxford University Hospitals, University of Oxford research laboratories, while some blood samples will be shipped to the GSK Vaccine Institute for Global Health (GVGH), Siena, Italy or delegated laboratories based in Italy. We would also send some samples to other researchers working with us on this research project, including researchers outside the European Union. Your samples will be identified by a code instead of your name and the link between your name and the code number will not be shared with external companies and laboratories.

If you choose to take part in this study, we will be asking for your separate permission to store blood (including cells and DNA) samples, in a collection of samples called Biobank. Details of this will be provided in a separate booklet after you are enrolled into this study, and you are free to say no to the Biobank and continue to take part in this study if you wish. If you do not wish for your samples to be stored in the Biobank, they will be destroyed 12 months after the last participant has completed the study.

What if any of my test results were abnormal?

If there are any abnormal results or undiagnosed conditions found in the course of the study these would be discussed with you and, if you agreed, your GP would be informed of these results. We would not report them to anyone else without your permission. For example, a new diagnosis of high blood pressure might be made. Any newly diagnosed conditions would be looked after by your GP.

Reimbursement

There are no costs for you to participate in this study. Study participants would be reimbursed for their time, travel and inconvenience of taking part in the study. The maximum reimbursement for any volunteer who completes the whole study is £585. All participants will be reimbursed based on the following figures:

Travel expenses: £15 per visit
Inconvenience of blood tests: £10 per blood donation
Time required for visits: £20 per visit

The sum reimbursed is on a pro rata basis, so, if for example, you choose to withdraw halfway through the study we would calculate your reimbursement based on the visits you have attended and samples that have been obtained.

Payments are made directly by bank transfer in instalments during the study. For this reason, we would require participants to provide their bank details at screening. Bank details would be kept confidential. Personal information such as your name, bank details and national insurance number may be shared with the University finance team to process or verify your reimbursement payments. Financial auditors may also audit the records where this information is held. All confidential data will be stored according to the UK General Data Protection Regulation (see below).

You may also receive reimbursement for any unscheduled visits you attend (if you have symptoms from the vaccine and need to be assessed). You would be reimbursed £45 per unscheduled visit, up to a maximum of £135 (equivalent of 3 unscheduled visits). If you do not require any unscheduled visits, you will not be reimbursed for this amount.

Would my taking part in this study be kept confidential?

Yes. All information that is collected about you during the course of the research would be coded with a study number and kept strictly confidential. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. You can search this website at any time. The website will include a summary of the research study results, but it may be many years before research results are posted. Any information about you that leaves the clinic would have any identifiable information removed so that you could not be recognized, with the exception of letters sent to your own GP. In order to enrol into this study, you would be required to sign a form, documenting that you consent for us to contact your GP. This is to inform him/her that you would be entering the study, and to ensure there are no medical reasons that would prevent you from taking part in this study. No one else would be told that you are involved in the study. We would only notify your GP of the results from any medical tests we performed with your permission.

Your information would be stored on a secure server, and paper notes would be held by the Oxford Vaccine Group in a locked cabinet. Your data is retained in case we need to contact you regarding any study related matters or if you wish to contact us regarding your participation in the study. We may also contact you to inform you of future related studies.

Responsible members of the University of Oxford and the Oxford University Hospitals NHS Foundation Trust may be given access to data for monitoring and/or audit of the study to ensure that the research is complying with applicable regulations. In addition, the following groups may inspect the study records without violating your confidentiality:

- Monitors who check that the study is being conducted to a high standard, including the Data and Safety Monitoring Committee (DSMC), an independent panel of experts responsible for trial safety and the Medicines and Healthcare Products Regulatory Agency (MHRA).

Coded data and samples would be sent to other researchers working with us on this research project, including researchers outside the European Union. Please note that your blood samples contain cells and DNA. Your DNA is unique to you so it can never be completely anonymous.

What will happen to my data?

Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' The University of Oxford is the data controller and is responsible for looking after your information and using it properly.

We will be using information from you and your medical records in order to undertake this trial and will use the minimum personally identifiable information possible. We will keep identifiable information about you such as contact details for a minimum of 5 years after the trial has finished. The need to store this information for longer in relation to licensing of the vaccine will be subject to ongoing review.

Paper notes will be held by the Oxford Vaccine Group in a locked cabinet. Once the trial has been completed, all documents, including personally identifiable data, would be archived in a secure facility, for a minimum of 5 years. Storage of this data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. If you complete online or telephone screening, and do not progress to in-person screening, your data will only be stored until the end of the trial.

If you have agreed that samples can be retained for future research then your personally identifiable information will be kept with restricted access solely for the purposes of sample management for a minimum of five years after the last sample has been either used or disposed of in order to meet regulatory requirements. Samples will be provided for future research only in a form that does not identifies you. We store research data securely at the University of Oxford indefinitely following removal of identifiable information.

The trial team will use your name and contact details, to contact you about the clinical trial, and make sure that relevant information about the trial is recorded for your care, in relation to your health during the trial and to oversee the quality of the trial. At the completion of the trial, unless you consent otherwise (e.g. if you request to be informed of other trials),

your personal details will not be used to contact you other than exceptional circumstances concerning your safety.

If you consent to take part in another trial carried out by the Oxford Vaccine Centre, we will retain a copy of your consent form until such time as your details are removed from our database but will keep the consent form and your details separate. Personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

Your bank details will be stored for 7 years in line with university financial policy.
Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Professor Andrew J Pollard, or his successor, as Director of the Oxford Vaccine Group will have the responsibility for custody of the data.

Further information about your rights with respect to your personal data is available at:
https://compliance.web.ox.ac.uk/individual-rights

If you withdraw from the trial, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.

You can find out more about how we use your information by contacting Oxford Vaccine Group on 01865 611400 or email info@ovg.ox.ac.uk.

What will happen at the end of the research study?

The results of the research will be published in a scientific medical journal and potentially presented at future conferences; this can potentially take a few years. All OVG publications will appear on the OVG website and you will receive a letter containing these results. Your individual results would not be identifiable, nor would you be identified in any report or publication. The results of the research will also potentially be used for future academic research within the Oxford Vaccine Group. Some of the research being undertaken will also contribute to the fulfilment of an educational requirement (e.g. a doctoral thesis).
Once the last laboratory test is performed in the study, all samples will be destroyed, unless you have consented for them to be transferred to the Biobank. If your samples are going to the Biobank, a copy of your informed consent form (which contains your personal information), are stored with those samples.

What if there is a problem?

If you have private medical insurance, you are advised to contact your insurance company before participating in this trial. The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this study.

Where can I get advice on whether to take part?

We are happy to answer any questions you might have and contacting us does not commit you to taking part in the study.

Other useful links for general information on taking part in research include:

- www.crn.nihr.ac.uk/can-help/patients-carers-public/how-to-take-part-in-a-study/
- www.nhs.uk/Conditions/Clinical-trials/Pages/Introduction.aspx

What if I wish to complain?

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Professor Andrew Pollard, Director of the Oxford Vaccine Group, (Tel: 01865 611400, Email: info@ovg.ox.ac.uk) or you may contact the University of Oxford Research Governance, Ethics and Assurance (RGEA) office on 01865 (6)16480 or the Head of RGEA, email ctrg@admin.ox.ac.uk.

At any time during the study you would be entirely free to change your mind about taking part, and to withdraw from the study. This would not affect your subsequent medical care in any way.

Who is funding the study?

The study is funded by an EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant, as part of a wider project to progress the iNTS-GMMA vaccine initially through the SALVO clinical trial with a further study to take place in sub-Saharan Africa. For further information on the Vacc-iNTS project please see: https://vacc-ints.eu

Independent monitoring of the study will be undertaken by Appledown Clinical Research Ltd which will be funded by GSK Vaccine Institute for Global Health (GVGH).

Who has reviewed and approved this study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given a favorable opinion by South Central - Oxford A Research Ethics Committee. In addition, this study has been reviewed by the Medicines and Healthcare Regulatory Agency (MHRA) the UK agency responsible for ensuring that medical products under investigation (in this case the active vaccine) is safe and appropriate to continue to clinical trial.

In summary, what would happen if I decide to take part in the study?

- We would ring you to check it is appropriate to include you in the study.
- You would then attend a screening visit in our department (CCVTM).
- At the screening visit we would go through the study in detail and answer any questions you may have. If you are happy to proceed, we would then ask you to sign a consent form. You would then have a brief medical assessment including a medical

history and physical examination. You would have a blood test and urine test (and a pregnancy test for women). These are to assess your eligibility for the study.

- Following satisfactory screening results confirming your eligibility we would invite you to the initial vaccination visit (V1) and enroll you into the study.
- You would be vaccinated with the study vaccine or placebo, once enrolled, then at month two and six.
- You would be seen two to four times after each vaccination; with a final visit one year after the first vaccination. These will include a blood test +/- an oral fluid sample.
- You would be required to fill in an eDiary for seven days after each vaccine.
- The study duration is approximately one year at which time you will be seen 12 times at the CCVTM (not including unscheduled visits or the initial screening visit).

What do I do now?

Thank you for considering taking part in this study. You do not need to make a final decision straight away. If you wish to discuss any element of the study further, then please contact us by either

- telephone **01865 611400**
- website: http://trials.ovg.ox.ac.uk/trials/salvo
- email: info@ovg.ox.ac.uk

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supp Material – Trial Summary
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Supp Material

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Supp Material
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Supp Material
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Supp Material
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	5

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Supp Material
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Supp Material
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5 Supp Material
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Supp Material

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6 Supp Material In Blinding and Randomisation Plan
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Supp Material In Blinding and Randomisation Plan
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Supp Material In Blinding and Randomisation Plan
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Supp Material In Blinding and Randomisation Plan
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Supp Material In Blinding and Randomisation Plan
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	Supp Material

		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Supp Material
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Supp Material
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8 Supp Material
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8 Supp Material
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8 Supp Material

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Supp Material
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Supp Material
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Supp Material
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Supp Material
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Supp Material
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Supp Material
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Supp Material
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	Supp Material

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Supp Material
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Supp Material
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA – no intended use of professional writers
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Protocol – as part of submission
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supp Material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp Material

BMJ Open

Salmonella Vaccine Study in Oxford (SALVO) Trial: Protocol for an Observer-Participant Blind Randomised Placebo-Controlled Trial of the iNTS-GMMA Vaccine within a European Cohort

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072938.R1
Article Type:	Protocol
Date Submitted by the Author:	17-Aug-2023
Complete List of Authors:	Hanumunthadu, Brama; Oxford University, Oxford Vaccine Group Kanji, Nasir; Oxford University, Oxford Vaccine Group Owino, Nelly; Oxford University, Oxford Vaccine Group Ferreira Da Silva, Carla; Oxford University, Oxford Vaccine Group Robinson, Hannah; University of Oxford Oxford Vaccine Group, Department of Paediatrics; NIHR Oxford Biomedical Research Centre White, Rachel; Oxford University, Oxford Vaccine Group Ferruzzi, Pietro; GSK Vaccines Institute for Global Health Nakakana, Usman; GSK Vaccines Institute for Global Health Canals, Rocio; GSK Vaccines Institute for Global Health Pollard, Andrew; University of Oxford, Paediatrics Ramasamy, Maheshi; Oxford University, Oxford Vaccine Group Vacc-iNTS Consortium, Collaborators; Vacc-iNTS Consortium
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Global health
Keywords:	Clinical Trial, IMMUNOLOGY, Public health < INFECTIOUS DISEASES

Salmonella Vaccine Study in Oxford (SALVO) Trial: Protocol for an Observer-Participant Blind Randomised Placebo-Controlled Trial of the iNTS-GMMA Vaccine within a European Cohort

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Keywords:
Clinical Trial
Non-typhoidal Salmonella
Vaccine
Invasive salmonellosis

Abstract

Introduction

Invasive non-typhoidal Salmonellosis (iNTS) is mainly caused by *Salmonella enterica* serovars Typhimurium and Enteritidis and is estimated to result in 77,000 deaths per year, disproportionately affecting children under 5 years of age in sub-Saharan Africa. Invasive non-typhoidal *Salmonellae* serovars are increasingly acquiring resistance to first line antibiotics, thus an effective vaccine would be a valuable tool in reducing morbidity and mortality from infection. While NTS livestock vaccines are in wide use, no licensed vaccines exist for use in humans. Here, a first-in-human study of a novel vaccine (iNTS-GMMA) containing *S.* Typhimurium and *S.* Enteritidis Generalized Modules for Membrane Antigens (GMMA) outer membrane vesicles is presented.

Method and Analysis

The *Salmonella* Vaccine Study in Oxford (SALVO) is a randomised placebo-controlled participant-observer blind phase 1 study of the iNTS-GMMA vaccine. Healthy adult volunteers will be randomised to receive 3 intramuscular injections of the iNTS-GMMA vaccine, containing equal quantities of *S.* Typhimurium and *S.* Enteritidis GMMA particles adsorbed on Alhydrogel, or an Alhydrogel placebo at 0-, 2- and 6-months. Participants will be sequentially enrolled into 3 groups: Group 1, 1:1 randomisation to low dose iNTS-GMMA vaccine or placebo; Group 2, 1:1 randomisation to full dose iNTS-GMMA vaccine or placebo;

Group 3, 2:1 randomisation to full dose or lower dose (dependant on DSMC reviews of Group 1 and 2) iNTS-GMMA vaccine or placebo

The primary objective is safety and tolerability of the vaccine. The secondary objective is immunogenicity as measured by O-antigen based enzyme linked immunosorbent assay. Further exploratory objectives will characterise the expanded human immune profile.

Ethics and Dissemination

Ethical approval for this study has been obtained from the South Central - Oxford A Research Ethics Committee (Ethics REF:22/SC/0059). Appropriate documentation and regulatory approvals have been acquired. Results will be disseminated via peer reviewed articles and conferences.

Registration Details

EudraCT Number: 2020-000510-14

Article Summary

Strength and Limitations

- SALVO is a first in human study of a novel vaccine against invasive non-typhoidal Salmonellosis (iNTS), a neglected disease largely affecting low- and middle-income countries.
- This study provides the opportunity to investigate the adaptive immune response to bacterial outer membrane antigens, supporting future vaccine development efforts against this disease.
- The volunteers recruited to this trial may not be immunologically representative of the ultimate target population in endemic areas. In addition the vaccination schedule in this study is based on the expected schedule in infants.
- In the absence of a known correlate of protection against iNTS disease, it will not be possible to determine vaccine efficacy in this trial.

Introduction

Non-typhoidal *Salmonellae* (NTS), such as *Salmonella enterica* serovars Enteritidis and Typhimurium, most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens (1). However, some NTS bacterial strains can also cause an invasive syndrome with bacteraemia, high fevers, and metastatic infection which if untreated can lead to septicaemia and death. Invasive non-typhoidal *Salmonella* (iNTS) infections are more common in children, the elderly and in the immunosuppressed, including HIV-infected individuals (2, 3).

The Global Burden of Disease study estimates 535,000 annual cases of iNTS globally, associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella* (1, 4). The highest burden of iNTS disease globally occurs in sub-Saharan Africa, with a pooled annual incidence of 52/100,000 (1, 4). This is likely an underestimate given the limited availability of diagnostics in the region coupled with non-specific disease presentation. The age at which infection occurs shows a bimodal distribution in most African studies with 68.3% occurring in children under 5 years and a second peak in the 30-40 years age group, believed to be associated with HIV, malaria, and malnutrition(3, 5, 6, 7).

Of the invasive pathogens responsible for iNTS in sub-Saharan Africa, *S*. Typhimurium is implicated in approximately two-thirds of all cases, with the ST313 serotype accounting for most isolates (8). In contrast to other non-invasive strains, African ST313 isolates often exhibit genomic degradation and pseudogene formation like that seen in typhoidal *Salmonellae*, which contribute to human host restriction and an invasive phenotype (9, 10, 11, 12). Furthermore, iNTS strains such as ST313 have been associated with multi-drug resistance, leading to *Salmonellae* being classified as WHO high priority antibiotic resistant pathogens (13, 14, 15).

High mortality, logistical difficulties in diagnosing infection in the developing world and increasing antimicrobial resistance strongly advocate for the development of an effective vaccine.

There are no currently licensed vaccines for iNTS although multiple candidates are in early phase development, including O-antigen (OAg) conjugates, oral attenuated vaccines, and multiple antigen display protein-polysaccharide conjugate vaccines. A trivalent iNTS-typhoid vaccine is currently in phase 1 (16).

The investigational product in this study is the iNTS-GMMA vaccine. This novel vaccine developed by GSK Vaccines for Global Health (GVGH) consists of outer membrane vesicles or Generalized Modules for Membrane Antigens (GMMA) of the two most common serotypes associated with invasive disease, *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) (17). Generalized Modules for Membrane Antigens (GMMA) particles contain several immunodominant antigens including the OAg component of bacterial lipopolysaccharide and outer membrane proteins. iNTS-GMMA are immunogenic in animal models, eliciting antibodies directed against OAg and demonstrating serum bactericidal

activity. Immunised animals also appear to have lower systemic bacterial loads on subsequent challenge (18).

This is the first trial to investigate the iNTS-GMMA vaccine in humans. Demonstration of safety and immunogenicity in this study will lead to progression to subsequent studies in a sub-Saharan country of high endemicity.

Study aims and objectives

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine and study the immune response to vaccination. Primary, secondary, and exploratory objectives are detailed in Table 1.

As this is a first-in-human vaccine trial, the primary objective is the safety and tolerability of the iNTS-GMMA vaccine which will be ascertained by the collection of solicited and unsolicited adverse events, serious adverse events, withdrawals and laboratory parameters. Solicited adverse events will be collected up to seven days following each vaccination and include local injection site reactions and systemic symptoms. Unsolicited adverse events will be collected up to 28 days following each vaccination, and outside of this period will be recorded only if medically attended. Serious adverse events and withdrawals will be recorded throughout the study. Laboratory adverse events will be recorded by obtaining blood samples to analyse full blood count, renal and liver profile at Days 0, 7, 28, 56, 63, 84, 168, 175 and 196.

The secondary objective is the determination of immunogenicity of the iNTS- GMMA vaccine. Immunogenicity will be measured using SEn and STm O-antigen specific enzyme-linked immunosorbent assays (ELISA) at timepoints Days 0, 7, 28, 56, 63, 84, 168, 175, 196 and 350.

Objective		Outcome measure
Primary	To determine the safety and tolerability of the iNTS-GMMA vaccine	Clinical review and participant recording of solicited, unsolicited adverse events, serious adverse events, withdrawals, and laboratory parameters (haematology/biochemistry)
Secondary	To investigate immunogenicity of the iNTS-GMMA vaccine	Measurement of serovar specific SEn and STm O-antigen by ELISA before and after vaccination
Exploratory	To further characterise the immune response to vaccination	Exploratory immunological analyses including functional antibody assays and antigen-specific memory B cell responses and T cell responses before and after vaccination

Table 1 Primary, secondary, and exploratory objectives and outcomes of the SALVO study.

Methods

Trial interventions: IMP and placebo

The iNTS- GMMA vaccine consists of outer membrane vesicles or GMMA from the two most common serovars causing invasive disease, *Salmonella* Enteritidis and S*almonella* Typhimurium adsorbed onto Alhydrogel (0.35 mg AL^{3+} /0.5 mL dose) and suspended in isotonic phosphate buffered saline. The parent bacteria have been genetically modified to increase production of outer membrane vesicles (Δ*tolR*) and reduce the toxicity of lipid A component within the lipopolysaccharide (Δ*msbB* and Δ*pagP*). The GMMA particles are filtered and purified to form the active component of the vaccine (19, 20, 21). Two dose levels will be used for this study: a full dose of 20 µg STmGMMA + 20 µg SEnGMMA (total 40 µg O- antigen); and a lower dose consisting of 5.3 µg STmGMMA + 5.3 µg SEnGMMA (total 10.6 µg OAg). The placebo matches the vaccine matrix and consists of Alhydrogel without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

Study design and setting

This is a first-in-human randomised placebo-controlled participant-observer blind trial of the iNTS-GMMA vaccine in healthy adults aged 18-55 years in the United Kingdom. A total of 30-42 participants will be randomised to receive 3 intramuscular doses of active vaccine or placebo at 0, 2 and 6 months (Figure 1). For further details please see SALVO Protocol in Supplementary Material 1.

Figure 1 SALVO vaccine and visit schedule

Participants will be sequentially enrolled into three groups (Figure 2) with a dose escalation between Group 1 (lower dose iNTS-GMMA vaccine, 10.6 µg total OAg content) and Group 2 (full dose iNTS-GMMA vaccine, 40 µg total OAg content). These first two groups will each consist of six participants who will be randomised 1:1 to the active vaccine or placebo. An additional six participants may be recruited to each of these groups if further safety information is required. Group 3 consists of 18 participants randomised 2:1 to receive the iNTS-GMMA vaccine or placebo. The decision to proceed to low or full dose vaccine in Group 3 will be based on safety reviews of Groups 1 and 2. There will be external safety monitoring reviews by the Data Safety Monitoring Committee (DSMC) between the two dose escalation groups and at a further two time-points in group three.

Figure 1 SALVO study design

Randomisation

Randomisation of participants will be carried out by unblinded study staff who are independent from the blinded team and do not perform any post vaccination procedures (such as ongoing eligibility or safety review). A web-based randomisation system will be used.

Blinding

This study will be conducted observer- and participant-blind from the time of randomisation until participant unblinding which will occur once the last participant has completed their final visit. Observer and participant blinding is required to minimise the risk of bias on the reporting of adverse events following the administration of vaccine.

Study visits

Vaccine or placebo will be administered at 0, 2 and 6 months. Participants will be directly observed for a minimum of 60 minutes following vaccination and then asked to complete an e- diary of their symptoms daily for 7 days following each vaccination. An in-person post-vaccination review will occur 7 and 28 days following each vaccine when participants will be reviewed for any possible adverse events. There are a total of 12 scheduled study visits and participants will be followed up for 1 year following first vaccination.

Recruitment and eligibility

Potential participants may be contacted by media advertisements, direct mail out or social media using an approved invitation letter or other approved advertising material to invite them to participate in the study. Participants will be reimbursed for their time, travel, and inconvenience. Healthy adults between the ages 18-55 years inclusive will be eligible for enrolment. Individuals will be initially screened for eligibility by telephone followed by face-to-face visits at the trial centre. Screening visits will involve obtaining informed consent (See SALVO Informed Consent Form in Supplementary Material 2), application of inclusion and exclusion criteria (summarised in Table 2) and clinical eligibility assessments including vital signs, physical examination, baseline blood tests, and urinalysis (SALVO Protocol in Supplementary Material 1).

Table 2 Summary of SALVO inclusion and exclusion criteria

Inclusion criteria	Willing and able to give informed consent for participation in the study
	Aged between 18-55 years inclusive
	In good health as determined by: - Medical history - Physical examination - Laboratory assessment - Clinical judgement of the investigators
	Willing to use highly effective contraception from one month prior to receiving the first vaccine and for the duration of the study (Females)
	Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
	Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study
	Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS)
	History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators.
	Have any known or suspected impairment or alteration of immune function.
	Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing

Exclusion criteria	Prior history of receipt of an oral typhoid vaccine (e.g.: Ty21a) within the last three years or a paratyphoid vaccine (as part of a clinical trial)
	Prior history of participation in a Typhoid or Paratyphoid controlled human infection study
	Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
	Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (except for COVID-19 vaccines)
	History of allergy or anaphylaxis to a previous vaccine or vaccine components
	Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
	Participation in another research study involving an investigational product or that which may compromise the integrity of the study
	Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
	Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study
	Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m2
	Any other significant disease or disorder which, in the opinion of the Investigator, may: - Put the participants at risk because of participation in the study - Influence the result of the study - Impair the participant's ability to participate in the study

Sample Size and Statistical Analysis

The sample size in this study is 30-42 participants to account for additional participants to be recruited on DSMC advice. As the initial phase 1 trial primary objective is safety and tolerability this sample size has been chosen to evaluate early data on adverse events associated with increasing dose-level, with a larger subsequent phase 1 trial in a highly endemic country planned dependant on the trial safety data. The statistics for the primary endpoint are descriptive, with no testing of statistical significance. The confidence intervals will be set at 95%.

Ethics and Dissemination

As a first-in-human vaccine trial the iNTS-GMMA vaccine has undergone appropriate pre-clinical toxicology studies indicating a well-tolerated vaccine. Participants will be actively monitored for their safety during the trial by review of an electronic diary, visits, clinical observations, and safety blood tests and will have access to a 24-hour medical contact number. Appropriate risk and benefits of the study will be communicated to the participants, and informed consent will be taken prior to any study related procedures. Local and national guidelines on confidentially and data protection will be adhered to.

The DSMC consisting of an experienced group of clinicians and a statistician will be appointed to provide real-time independent oversight of safety and trial conduct. The DSMC will review safety data collated from participant and clinician recorded entries including solicited and unsolicited adverse events, laboratory results and vital signs. Progression of enrolment from Group 1 to Group 2 to Group 3 will only occur after DSMC review of the relevant safety data. Further DSMC reviews will occur regularly throughout the trial. A

development safety update report (DSUR) for the IMP will be prepared annually, on the anniversary of the MHRA approval for the trial.

Undertaking non-COVID-19 research during a dynamic COVID-19 pandemic represents a major logistical challenge. The safety of participants remains paramount and good infection prevention and control practices will be followed throughout the trial. The SALVO study team will monitor cases of COVID-19 within the participant cohort and the local population and will follow current national guidelines on COVID-19 with options including switching to phone appointments, halting, or extending the trial.

Once the trial has been completed including analysis of data, results will be published in a peer-reviewed journal and presented at conferences. The results of this study will directly impact the appropriateness of subsequent trials with a larger sample size to begin in a sub-Saharan country of high iNTS endemicity.

This study has been approved by South Central - Oxford A Research Ethics Committee on 28th April 2022.

Patient and public involvement statement

The protocol, study information booklet and recruitment materials were reviewed by a local patient consultation group who provided feedback and comments on the initial documents. Their comments led to changes in the participant-facing documents, ensuring they are easy and clear for participants to understand (Please see SALVO Participant Information Sheet in Supplementary Material 3).

Study Dates

Study recruitment began in May 2022. The estimated last participant last visit (LPLV) will be in December 2023.

Discussion

This will be the first phase 1 study investigating the iNTS-GMMA vaccine. The data generated by this trial will guide future vaccine development using this GMMA technology and may contribute to the licensure of the first vaccine against invasive non-typhoidal *Salmonella* species.

iNTS disproportionately causes severe disease in children under the age of 5 years in sub-Saharan Africa, and an effective vaccine will be of greatest benefit in this vulnerable population. As the UK is a country with a low burden of non-typhoid *Salmonella* disease (22, 23) healthy adult volunteers recruited to the SALVO study will be unlikely to have pre-existing immunity to *S*. Typhimurium or *S*. Enteritidis. However, assessment of the immunogenicity of the iNTS-GMMA vaccine in an immunologically naïve cohort in SALVO will inform the decision to progress to a second larger phase 1 trial in a sub-Saharan African adult population where iNTS is endemic. This second study may provide insight into the

vaccine-induced immunogenicity following pre-existing immunity in adults. Future studies will recruit African infants, in whom any future licensed vaccine is likely to be deployed. However without a known immune correlate of protection, the efficacy of the vaccine in prevention of iNTS disease cannot be determined without much larger field studies in endemic settings.

As GMMAs originate from the bacterial outer membrane they contain both the immunodominant OAg as well as multiple membrane proteins in their native conformation(19). Alongside measurement of OAg binding antibody after vaccination, this study will use functional antibody assays (including serum bactericidal activity), to interrogate the outer membrane protein specific responses. This may reveal conserved proteins present across multiple *Salmonella* serovars capable of eliciting pan-protective immune responses. As a first-in-human phase I trial, the study design prioritises the safety of the participants. The dose escalation design allows investigation of the safety of the vaccine in a small cohort and at a significantly lower dose prior to escalation to the full dose vaccine. Multiple DSMC and internal safety reviews at set intervals provide further mandatory time points to formally review the safety data in addition to real-time monitoring by the study team. This is a participant-observer blind, randomised, placebo-controlled study. Blinding of the trial aims to reduce both recall and observational bias, which is intended to allow confidence in the study's final outcome. The use of a placebo-controlled study design allows a direct comparison between active vaccine and placebo groups aiming to account for potentially confounding factors, such as the incidence of SAR-Cov-2 infection during the study.

SALVO will be the first clinical trial of the iNTS-GMMA vaccine. Currently, the only other iNTS-based vaccines to enter clinical trials are two trivalent vaccines covering *S. enterica* serovars Typhimurium, Enteritidis and Typhi (24, 25), with results to be published. A live attenuated oral *S.* Typhimurium was trialled in participants in 2009 but has not progressed further in the intervening years (26). Further iNTS-based vaccines are progressing through the pre-clinical phase including flagellin and OmpD-based vaccines (27).

The study protocol was prepared in accordance with the SPIRIT 2013 Checklist (28).

Authors' contributions

BH and MR designed and authored the protocol. BH and NK wrote the manuscript. NO, CF, HR, RW, PF, UN, RC, AJP contributed to the protocol design and/or study set up.

Conflicts of Interests

The iNTS-GMMA vaccine has been provided by the GSK Vaccines Institute for Global Health (GVGH). GVGH has reviewed the protocol developed by the Oxford Vaccine Group, University of Oxford and provided funding for clinical trial monitoring.

PF, UN and RC were employees of the GSK Vaccines Institute for Global Health at the time in which the study was conducted. UN owns shares in GSK. GSK Vaccines Institute for Global Health Srl is an affiliate of GlaxoSmithKline Biologicals SA. This does not alter the authors' adherence to all Journal policies on data and material sharing.

Funding

This research was funded in whole or in part by EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission.

Data Statement

The Protocol Version 3.1 January 2023 has been published in supplementary material 1.

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Word Count 2636

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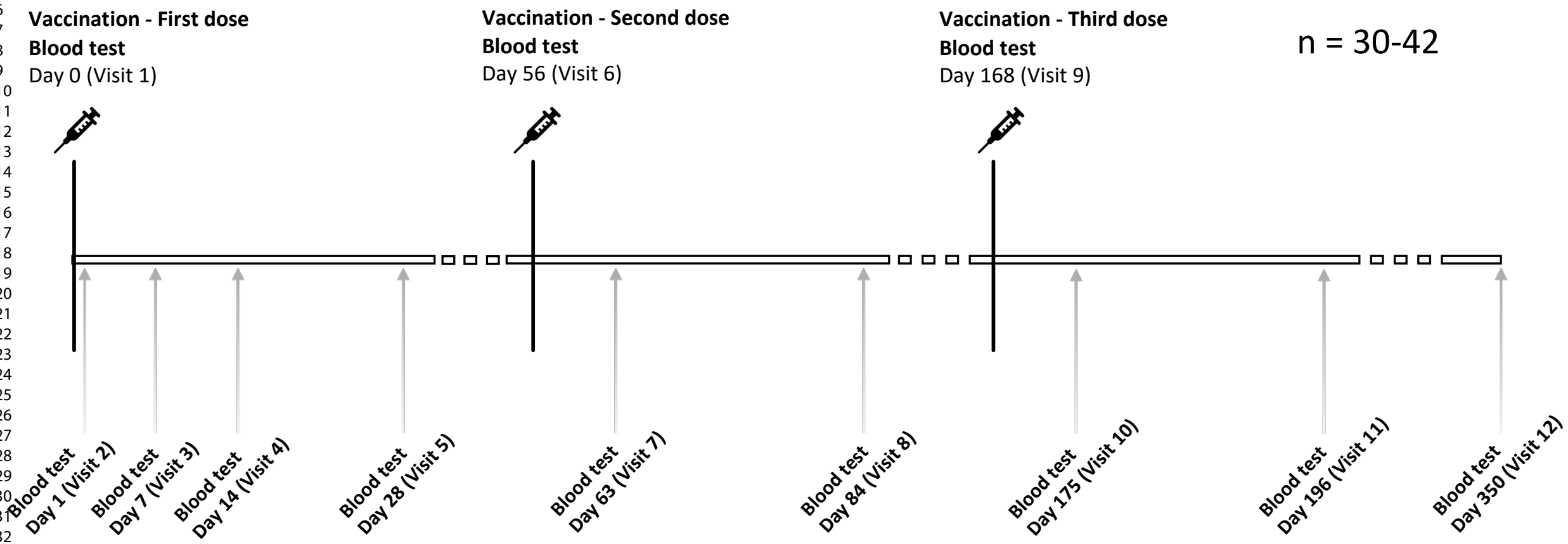
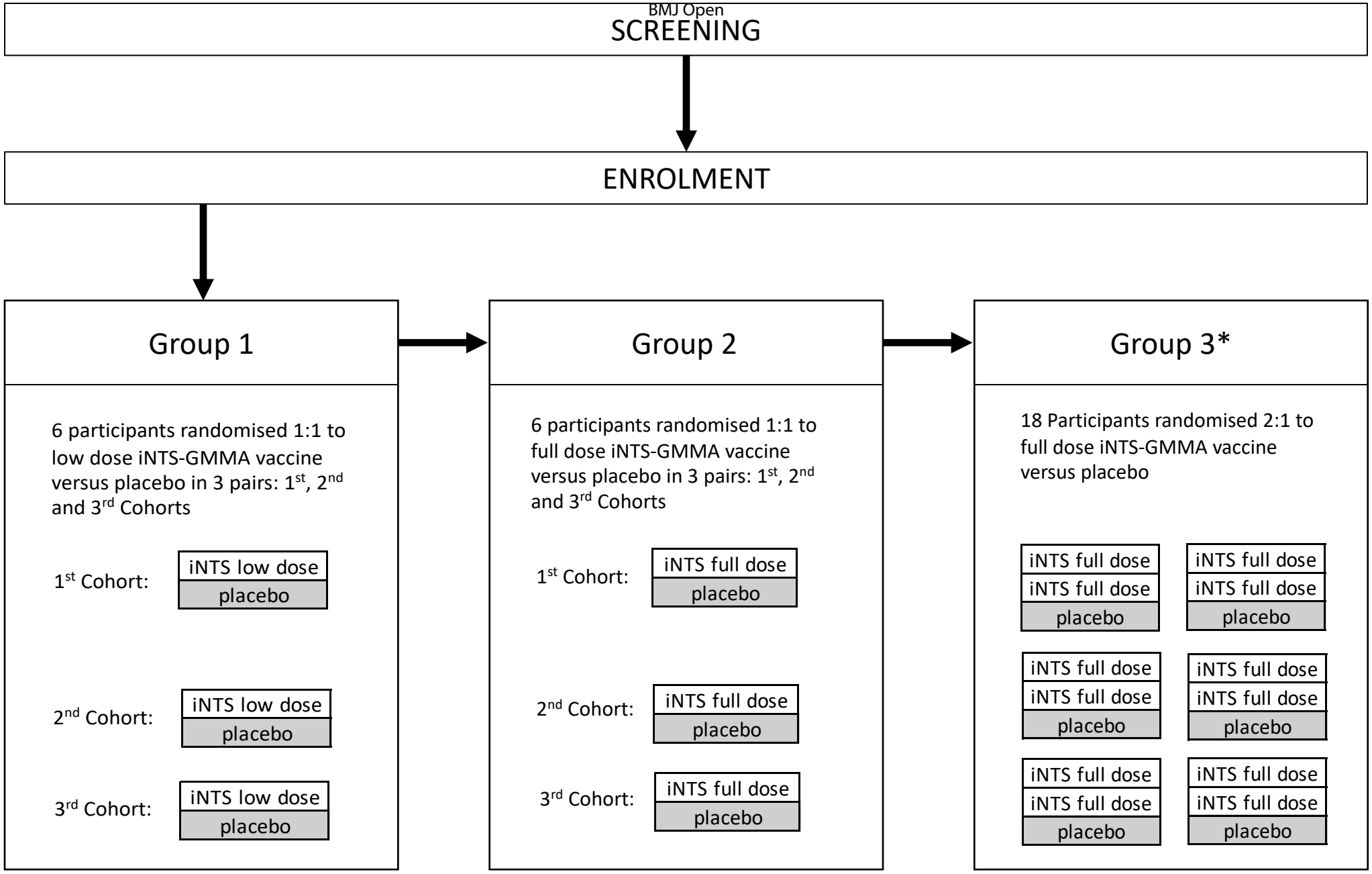

Vaccination - First dose
Blood test
Day 0 (Visit 1)
Vaccination - Second dose
Blood test
Day 56 (Visit 6)
Vaccination - Third dose
Blood test
Day 168 (Visit 9)
n = 30-42
Blood test Day 1 (Visit 2)
Blood test Day 7 (Visit 3)
Blood test Day 14 (Visit 4)
Blood test Day 28 (Visit 5)
Blood test Day 63 (Visit 7)
Blood test Day 84 (Visit 8)
Blood test Day 175 (Visit 10)
Blood test Day 196 (Visit 11)
Blood test Day 350 (Visit 12)



**Decision to proceed to low or full dose will be based on safety reviews of Groups 1 and 2.*

Trial Title: A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella

Internal Reference Number / Short title: OVG2020/01 Salmonella **V**accine Study in **O**xford (SALVO)
Ethics Ref: 22/SC/0059
IRAS Project ID: 1005098
EudraCT Number: 2020-000510-14
Date and Version No: 13 January 2023 / Version 3.1

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Sponsor: University of Oxford
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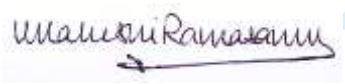
The GSK Vaccines Institute for Global Health (Monitoring Costs Only)

Chief Investigator Signature:



Statistician Signature:

We declare no financial conflict of interest in this study.

Confidentiality Statement
This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

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KEY TRIAL CONTACTS

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Funder(s)	EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439
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2 LAY SUMMARY

Nontyphoidal Salmonellae are types of bacteria that can cause gut infections resulting in diarrhoea, both in the UK and globally. However, under some circumstances, these bacteria can cause a more severe illness where infection spreads beyond the gut into the blood stream, a condition termed invasive non-typhoidal Salmonellosis (iNTS). iNTS disease is an under-recognised cause of disease and death in Sub Saharan Africa. In these regions, it primarily occurs in young children, particularly those with malaria and malnutrition. High death rates, difficulties in diagnosing this infection in the developing world, increasing resistance of the bacteria to common antibiotics, and spread via contaminated food and water make development of an effective and affordable vaccine against iNTS an essential control measure.

A new and innovative vaccine (iNTS-GMMA), has been developed which is based on the formation of bacterial outer surface particles. This vaccine facilitates exposure of components of the bacteria to the human immune system without the risk of causing infection. Developed by GSK Biologicals and GSK Vaccines Institute for Global health (GVGH), the aim of this vaccine is to confer immune protection to the most common African strains of the bacteria causing iNTS disease.

This study is a first-in-human clinical trial involving 30-42 healthy adult participants who will be randomly allocated to receive either iNTS-GMMA or a placebo. The main objective of this trial is to evaluate the safety of the iNTS-GMMA vaccine in healthy adults in the UK. The secondary objective is to investigate the human immune response to iNTS-GMMA vaccine.

3 SYNOPSIS

Trial Title	**A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella**
Internal ref. no. (or short title)	**OVG2020/01 Sal**monella **V**accine Study in **O**xford (SALVO)
Trial registration	ISRCTN51750695
Sponsor	University of Oxford Research Governance, Ethics and Assurance Joint Research Office Boundary Brook House Churchill Drive Headington Oxford OX3 7GB United Kingdom
Funder	EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS The GSK Vaccines Institute for Global Health (Monitoring Costs Only)
Clinical Phase	Phase I, First in Humans
Trial Design	Single centre, participant-observer blind, randomised placebo-controlled safety and immunogenicity interventional study
Trial Participants	Healthy adults, aged 18-55 years inclusive
Sample Size	• 30-42 participants. • Up to 12 participants randomised 1:1 to receive lower dose at 10.6µg (3.8 dilution of full dose) OAg of iNTS GMMA vaccine or a placebo. • Up to 12 participants randomised 1:1 to receive the full dose at 40µg OAg of iNTS GMMA vaccine or a placebo • Eighteen participants randomised 2:1 to receive either the lower dose or full dose dependant on DSMC review versus placebo.
Planned Trial Period	• Total trial period including data analysis is 36 months. • The intended duration of a participant on the trial is 12 months. • The total duration from first participants first visit to last participants last visit is maximum 18 months.
Primary Objective	To determine the safety and tolerability between two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each

	adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years inclusive when given three doses of vaccine at 0, 2- and 6-month intervals.
Secondary Objectives	To investigate the immunogenicity at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Exploratory Objectives	To further investigate the immunogenicity using exploratory immunological analyses at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Primary endpoint	The recording and assessment of local and systemic adverse events following administration of each vaccine dose: 1. Tenderness and pain at the injection site 2. Induration 3. Redness 4. Swelling 5. Headache 6. Malaise 7. Myalgia 8. Nausea and/or vomiting 9. Diarrhoea 10. Abdominal Pain 11. Anorexia 12. Arthralgia 13. Fatigue 14. Fever 15. Blood parameters (haematology / biochemistry) 16. Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR
Secondary endpoints	Immunological assays to study the immune responses to vaccines, including:

	1. Antibody concentration against serovar specific O antigen determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
Exploratory endpoints	Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to: 1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose. 2. Serum bactericidal antibody (SBA) titre against vaccine homologous strains before and after each dose 3. Serum bactericidal antibody (SBA) titre against a panel of other strains before and after each dose 4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose 5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose 6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose 7. Investigate the innate and adaptive response to the iNTS-GMMA vaccine by utilising next generation sequencing of the transcriptome to evaluate the differential gene expression profile and DNA storage for investigation of the genetic associations with the immune response 8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose 9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays 10. Faecal antibody concentration against O antigen determined by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection. 11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.
Investigational Medicinal Products	1. iNTS GMMA vaccine 2. Comparator (Placebo)
Form of vaccine	Glass vials containing 0.7ml sterile suspension containing either STmGMMA (80 µg/mL in OAg) or SEnGMMA (80 µg/mL in OAg) formulated with Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5

Form of Comparator (Placebo)	Glass vials containing 0.7ml sterile suspension containing Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH6.5
Dose	Lower Dose: 5.3 µg (OAg) STmGMMA/Alhydrogel + 5.3 µg (OAg) SEnGMMA/Alhydrogel (3.8x dilution of full dose generated by combining 0.5 mL of the two vaccine components into an empty vial and transferring 0.25 mL of the two components into 0.7 mL of the placebo vial, 0.5mL of combined vaccine to be administered) Full Dose: 20 µg (OAg) STmGMMA/Alhydrogel + 20 µg (OAg) SEnGMMA/Alhydrogel (generated by combining equal volumes of the two vaccine components in an empty vial, 0.5 mL of combined vaccine to be administered) Comparator (Placebo): 0.5mL to be administered
Route	Intramuscular
Vaccine Schedule	3 doses given at 0, 2 and 6 months

4 ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CCVTM	Centre for Vaccinology & Tropical Medicine
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DMSC	Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
ELISA	Enzyme Linked Immunosorbent Assay
GCP	Good Clinical Practice
GMMA	Outer membrane exosome from genetically modified Gram negative bacteria used as an antigen delivery system; GMMA is a pun on the Italian word for jewel or bud.
GP	General Practitioner
GVGH	GSK Vaccines Institute for Global health
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
iNTS	Invasive Non-typhoidal Salmonella
IRB	Independent Review Board
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
OVG	Oxford Vaccine Group
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PBMC	Peripheral Blood Mononuclear Cell
RGEA	Research Governance, Ethics and Assurance

R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SEn	*Salmonella* Enteritidis
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
STm	*Salmonella* Typhimurium
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TOPS	The Over volunteering Prevention System (http://www.tops.org.uk)

5 BACKGROUND AND RATIONALE

5.1 INVASIVE NON-TYPHOIDAL SALMONELLA DISEASE

Salmonella enterica is a rod-shaped Gram-negative bacterium that is further classified into approximately 2500 serovars, a number of which can cause human infection. Of these, *Salmonella* Typhi and Paratyphi are the causative agents of enteric fever and together are referred to as Typhoidal Salmonella. Non-typhoidal Salmonellae (NTS), such as *S.* Enteritidis and *S.* Typhimurium most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens. However, these organisms can also cause an invasive syndrome with bacteraemia, high fevers and metastatic infection which if untreated can lead to septicaemia and death. Invasive non typhoidal *Salmonella* (iNTS) infections are more common in resource poor settings of sub-Saharan Africa, children, the elderly and in the immunosuppressed, including HIV-infected individuals. Unlike *S.* Typhi and *S.* Paratyphi, whose only reservoir is humans, NTS can be acquired from multiple animal reservoirs including domestically farmed animals[1–3]. However, data from sub-saharan Africa appears to suggest that human-to-human transmission remains the primary mode of dissemination[4].

5.2 BURDEN OF DISEASE

The Global Burden of Disease Study estimates 535,000 annual global cases of iNTS; associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella*[5].

In the UK, NTS infection is a frequent cause of foodborne gastroenteritis outbreaks. Public Health England surveillance data showed that NTS was isolated from 8630 patient samples in 2016[6]. Whilst there are no human seroprevalence studies, it is clear that NTS remains endemic; particularly in livestock and poultry, with multiple veterinary programs implementing control methods such as testing and vaccination[7]. Although less common than in other parts of the world, invasive syndromes do also occur in the UK. Between 2004-2015, there were 2484 iNTS blood isolates in England, with neonates, individuals aged over 65 and men more likely to have a bacteraemia[8]. A case series of 82 iNTS blood cultures at a single site in the UK, found bacteraemias without an intestinal focus to be related to underlying immunosuppression in 80% of cases[9].

By far the highest burden of iNTS disease throughout the world remains in sub-Saharan Africa with 78.8% of global cases[5]. However, this is a likely underestimate of the true burden of disease, given the limited availability of diagnostics (blood cultures) in this setting. In addition, the clinical presentation of invasive disease in children is poorly defined, typically presenting as a febrile illness similar to malaria, enteric fever or pneumonia. Nevertheless, iNTS remains one of the most commonly identified causes of bacteraemia in the region[3]. A study in Malawi focused on adult medical admissions found iNTS to be the most common isolate from blood cultures, contributing to 37% of all bacteraemias in a 12 month period, and associated with a 33% case fatality rate[10]. A study in Ghana in a paediatric population found that 23.5% of bacteraemias were associated with *Salmonella* sp. of which 59% were associated with *Salmonella* Enteritidis[11]. Another paediatric single-centre study in Malawi found a case fatality rate of 20% in invasive *Salmonella* disease, of which 94% was associated with iNTS. The highest incidence was in the under three age group with 81% of cases, with a median age of 16 months[12].

The age at which infection occurs show a bimodal distribution in most African studies[2,13]. The majority of infections are in the under 5 age group with 68.3% of cases[13]; with a second peak in 30-40 years age group, which is believed to be associated with the higher incidence of HIV, malaria and malnutrition[2,13]. The Global Burden of Disease estimates a disproportionably high incidence of approximately 233,400 cases, including 31,630 deaths in the under 5 year olds globally in 2017[5].

In a meta-analysis of 22 African studies of all ages, *Salmonella* sp. was the most prevalent isolate in blood cultures at 29.4 %, with iNTS accounting for 58.4%. Of those patients who had additional HIV testing, iNTS was particularly associated with HIV infection (OR 8.2) compared with *Salmonella* Typhi bacteraemia (OR 0.07)[14]. In addition, studies have shown a decline in iNTS to be associated with a reduction in HIV, as a result of the availability of ART, as well as public health measures designed to minimise the impact of malaria and malnutrition[3,15,16]. *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) represent the most common serovars to cause invasive disease both globally and within sub-Saharan Africa. *Salmonella* Typhimurium isolates with the multi-locus sequence type (MLST) ST313 have emerged as a dominant subtype in sub-Saharan Africa. Genomic analysis of these isolates reveals genomic degradation and pseudogene formation, characteristics consistent with host adaptation and restriction by the bacterium. Along with enhanced invasive and virulence factors, the ST313 subtype is associated with multidrug resistance, potentially contributing to higher incidence and mortality of iNTS in sub-Saharan Africa[3,17]. In contrast, UK isolates are usually MLST ST19 and are commonly associated with gastroenteritis.

5.3 IMMUNO-PATHOGENESIS

It remains unclear why some infections result in local self-limiting disease whilst others cause invasive disease. In adults, iNTS is more common in the immunocompromised, including individuals with HIV, malaria, malnutrition, sickle cell disease and other immunodeficiency states, such as chronic granulomatous disease[2,3].

In children under 5 years of age, acquisition of functional antibody is associated with protection from *Salmonella* infection. Thus the highest peak of infection is between 6 months to 2 years of age, with the incidence declining thereafter. This observation correlates with protection from placentally transferred maternal antibody waning by 6 months and the development of detectable functional antibodies from natural exposure by 16 months of age[18,19]. A study in Malawi found detectable functional antibodies peaked at 35 months. In addition, this study found STm-specific CD4 T helper cells peaked at 14 months then declined, suggesting CD4 T helper cells alone were not protective.

Functional antibodies targeting iNTS can effect bacterial killing though activation of the classical pathway of complement and subsequent membrane attack complex assembly or by opsonisation, facilitating phagocytosis and oxidative burst-mediated intracellular killing. Possible targets for functional antibody include the O antigen (OAg) component of outer membrane lipopolysaccharide (LPS) or outer membrane protein antigens (OMP Ag) or flagella antigen (FliC). Human studies suggest that bactericidal activity correlated with the detectable levels of IgG to Lipopolysaccharide (LPS) but not to outer membrane protein (OMP) or flagella proteins. However antibodies against the FliC flagellar protein and membrane-bound porin proteins such as OmpD have been shown to be protective in mouse models[20,21].

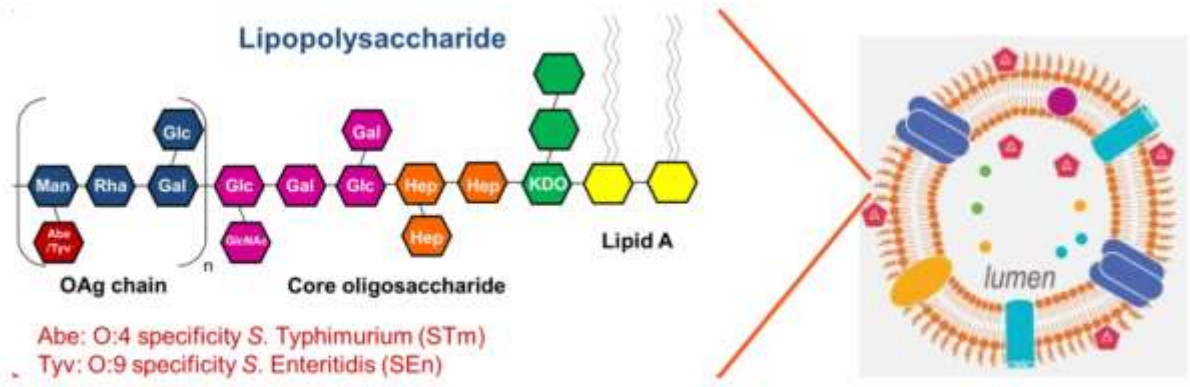


Figure 1.1 Diagram of O Antigen and Lipopolysaccharide constituent of GMMA (taken from Koeberling et al., 2017[22])

Together with lipid A, OAg forms part of the lipopolysaccharide attached to the outer membrane of Salmonella. OAg is the most immunodominant part of LPS and functions as a virulence factor with multiple studies showing attenuation of virulence in bacteria with mutations impairing OAg synthesis and longer OAg chains associated with increased resistance to antibody and complement mediated killing[23]. OAg structure can vary dependant on the individual oligosaccharide units, and this forms the basis of serotyping commonly used in microbiology laboratories. STm is associated with O:4,5 whilst SEn is associated with O:9[24].

5.4 PRIOR EXPERIENCE WITH iNTS VACCINES

The WT05 vaccine produced by Microscience Ltd. is the only iNTS vaccine candidate to date that has progressed to a phase one clinical trial. This dose escalation study randomised participants to receive either an oral attenuated *Salmonella* Typhi or an oral attenuated *Salmonella* Typhimurium at a dose 10^7, 10^8 or 10^9 CFU. Both strains were attenuated with mutations in aroC and ssaV, with ssaV associated with a reduction in function of the type III secretion system. Of the nine participants who received the *Salmonella* Typhimurium oral vaccine only the three participants in the 10^9 group had a significant antibody response[25]. There were no serious adverse events observed and each dose level of oral attenuated Salmonella *Typhimurium* appeared well tolerated. Other vaccines in development include an oral live attenuated vaccine (CVD 1931, CVD 1994), a bivalent OAg conjugate vaccine (COPS, flagellin) and protein vaccines (flagella and OmpD). All remain in the pre-clinical phase[26,27].

5.5 RATIONALE FOR VACCINE BASED ON GMMA TECHNOLOGY

The outer membrane of Gram-negative bacteria such as *Salmonella* naturally release outer membrane vesicles (OMVs) containing outer membrane proteins. OMVs have clear potential as vaccines, displaying immunogenic surface antigens in their natural conformation whilst avoiding the risks of potential infection associated with the use of live attenuated vaccines. Detergent-extracted OMVs from homogenised bacteria have been successfully licensed and used as vaccines against capsular group B meningococcal infections (MenBvac, Bexsero, VA-MENGOC-BC).

In contrast to traditional OMVs, the GMMA-technology derives outer membrane exosomes by induction of hyper blebbing from viable genetically modified bacteria. iNTS GMMA producing

vaccine strains are created from wild-type strains by deletion of *tolR*, *msbB* and *pagP* genes from *S.* Typhimurium and *S.* Enteritidis. Together, these modifications facilitate increased production of membrane blebs (Δ*tolR*) and reduced-acylation of the lipid A component of bacterial lipopolysaccharide (LPS via Δ*msbB* and Δ*pagP*). GMMA particles with hexa- or penta-acylated LPS induce less cytokine production *in vitro* by human peripheral blood monocytes compared to wild type GMMAs (containing hepta-acyltaled lipid A), which potentially reduces *in vivo* reactogenicity. Furthermore, GMMAs are potentially highly immunogenic, as they present O polysaccharide (OAg) and outer membrane protein antigens identified as immune targets in their native configuration, with minimal expression of cytoplasmic or inner membrane proteins. Purified GMMA particles are filtered, concentrated and implemented as a vaccine[28,29].

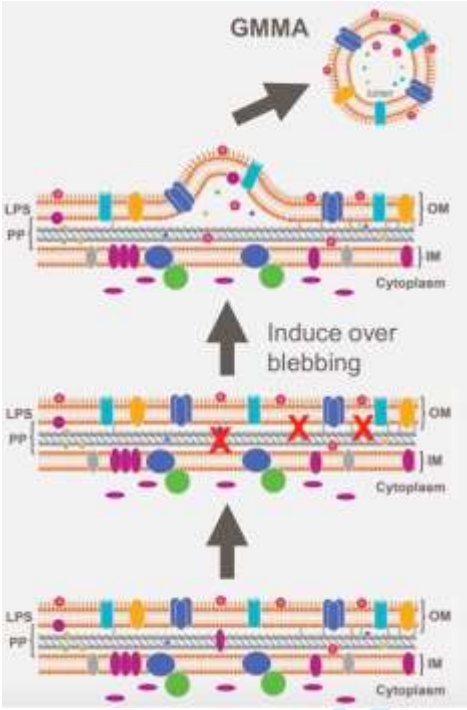


Fig 1.2 Outer membrane blebbing to create GMMA particles (taken from Koeberling 2017[22])

5.6 CLINICAL STUDIES OF ENTERIC GMMA VACCINES

This is the first trial to investigate a 2-component iNTS GMMA vaccine in humans. However, there have been four proof-of-concept clinical trials of a *Shigella* Sonnei GMMA vaccine; three phase 1 trials (including one phase 1 extension trial) in Europe[30,31] and one phase 2a trial in Kenya[32]. Over 100 participants have been enrolled, with all studies demonstrating a well-tolerated vaccine with an acceptable safety profile. The most common adverse event was mild to moderate injection site pain. A transient asymptomatic neutropenia was reported in 9.6% of participants in the two initial phase 1 European trials[30].

In vitro analysis of *Shigella* GMMA have revealed they contain > 95% of the outer membrane and periplasmic proteins[33]. By extrapolation this suggests the iNTS GMMA vaccine may contain a similar percentage of proteins which may contain multiple antigens including OAg and OMP Ag eliciting a potentially broadly protective immune response.

In Phase I trials, the *Shigella* GMMA vaccine used a 3-dose regime with intervals of 0, 1 and 2 months. The iNTS GMMA vaccine will use a 3-dose regimen at 0, 2 and 6 months to allow involution of B cell germinal centres and T cell responses between doses to better investigate

booster and memory responses. The highest *Shigella* GMMA vaccine dose so far tested contained approximately 6 µg OAg and 100 µg protein, while the highest iNTS GMMA dose will contain 40 µg OAg and approximately 31 µg protein.

THE INVESTIGATIONAL PRODUCT: 2-COMPONENT iNTS GMMA SALMONELLA VACCINE

5.7 DESCRIPTION OF INVESTIGATIONAL PRODUCT

The iNTS GMMA Vaccine consists of the outer membrane exosomes of the two most common serovars causing invasive disease, *Salmonella* Enteritidis (O:9) and S*almonella* Typhimurium (O:4.5), adsorbed to Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline pH 6.5. Two doses levels will be used for this study: a full dose of 20 µg STmGMMA as OAg and 20 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml full dose; and a lower dose at 3.8 dilution rendering a vaccine consisting of 5.3 µg STmGMMA as OAg and 5.3 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml dose. The placebo matches the vaccine matrix and consists of Alhydrogel (0.35 mg AL^{3+} / 0.5 mL dose) in isotonic 20mM Phosphate buffered saline pH 6.5 without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

5.8 CHARACTERISTICS OF VACCINE PREPARATION

STmGMMA and SEnGMMA, the iNTS vaccine active components, are formulated separately at 80 µg GMMA as OAg/ml batches with the GMMA absorbed onto 0.7mg Alhydrogel as Al^{3+}/ml Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline at pH6.5 . Each batch is aliquoted into vials containing 0.7 ml of either STmGMMA/ Alhydrogel or SEnGMMA Alhydrogel, stored at 2-8 ºC.

At the clinical site, 0.5ml of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel will be mixed in an empty vial using a standard operating procedure to yield 1ml of the 2-component iNTS GMMA vaccine containing 40 µg STmGMMA as OAg and 40 µg SEnGMMA as OAg and 0.7 mg Al^{3+} of Alhydrogel suspended in isotonic 20mM Phosphate buffered saline pH 6.5. This procedure including dilution for the lower dose iNT-GMMA vaccine is detailed in the clinical study plan and will be performed at the clinical site by trained study personnel. This procedure has been developed and evaluated by GVGH. Further stability testing has been performed to confirm the quality of the iNTS-GMMA vaccine for up to 6 hours post mixing, however it is envisaged that the final iNTS-GMMA vaccine will be used within 1 hour of mixing.

5.9 IMMUNOGENICITY IN MICE

Anti-OAg ELISA titres correlate with functional antibody assays consistent with immunity in children[18,19]. Mouse studies using challenge with either wild type *Salmonella* Typhimurium or with an OAg knockout strain higher levels of functional bactericidal antibodies in animals exposed to the bacteria expressing OAg [24].

Mice immunised either with STm and SEn GMMA vaccine showed both an elevated OAg titre by ELISA and complement mediated antibody killing via serum bactericidal assay (SBA). These results were then replicated in mice immunised with the two component (STm and SEn) iNTS GMMA vaccine (Figure 1.3) confirming the immunogenicity and validity of the bivalent

vaccine[34]. On challenge with either live *S.* Typhimurium or *S.* Enteritidis, immunised mice showed a reduction of bacterial burden (CFUs) in the spleen and liver.

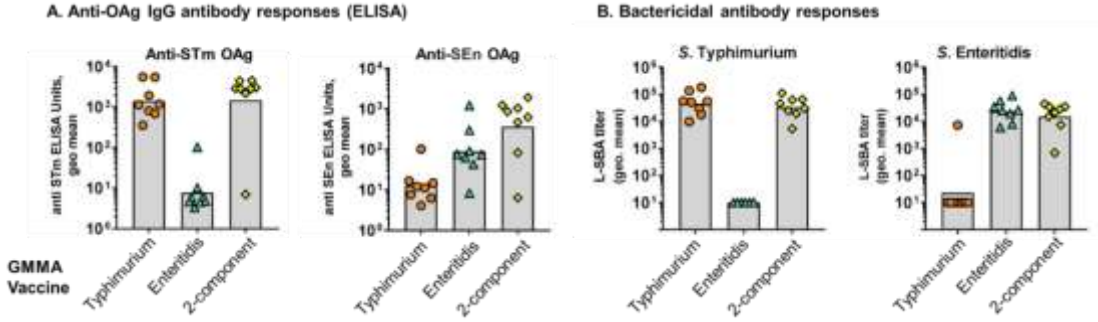


Figure 1.3 (A) Anti-STm and anti-SEn OAg IgG serum antibody responses (ELISA units) and (B) SBA responses against *S.* Typhimurium and *S.* Enteritidis strains. CD1 mice were immunized twice, four weeks apart with 0.16 µg (based on OAg) STmGMMA or SEnGMMA adsorbed on Alhydrogel or a mixture of 0.16 µg each of the two formulated GMMA (2-component iNTS-GMMA). Sera were collected two weeks after the second immunization. Symbols represent results from individual mice, bars represent group geometric mean titres.

In addition there was no significant difference in antibody response when the GMMAs were combined with Alhydrogel[34]

Study	Vaccine	Adsorbent	Dose [µg]	Anti-OAg IgG ELISA titre (geometric mean)
1	STmGMMA	None	2.5	61588
	STmGMMA	Alhydrogel	2.5	69572
2	SEnGMMA	None	2.5	8953
	SEnGMMA	Alhydrogel	2.5	10401

Table 1.1 Anti-OAg IgG ELISA units induced in mice by STmGMMA and SEnGMMA administered with or without Alhydrogel

5.10 IMMUNOGENICITY IN RABBITS

OAg is also a immune target in rabbits. Animals challenged with wild type *Salmonella* Typhimurium or OAg knockout strains develop higher bactericidal antibody response in the wild type arm[24].

In a rabbit immunogenicity study, STm GMMA/Alhydrogel, SEn GMMA/Alhydrogel and the 2-component iNTS-GMMA vaccine were well tolerated and induced strong anti-STm OAg and anti-Sen OAg serum IgG responses and high bactericidal antibody activity against *S.* Typhimurium and *S.* Enteritidis strains.

5.11 TOXICOLOGY

The dosing regimen has been estimated based on immunogenicity in mice and rabbits[24,34], in conjunction with the results of monocyte activation, pyrogenicity and repeat dose toxicology studies in rabbits.

In order to maintain immunogenicity whilst reducing reactogenicity, expression of Lipid A, a potent stimulator of the innate immune system, has been modified via deletions of the genes msbB and pagP. A monocyte activation model with human PBMC stimulated by a mixture of 1:1

STm and SEn iNTS GMMAs produced less IL-6 (a marker of inflammation) than wild-type GMMAs[24]. A similar cytokine release profile was stimulated by unformulated *S. sonnei* 1790-GMMA, which, as Alhydrogel formulated vaccine, were well tolerated in EU and Kenyan adults.

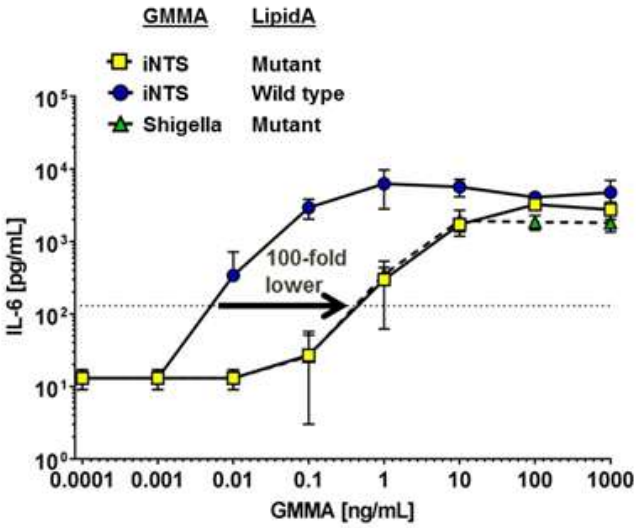


Figure 1.4 Monocyte activation test IL-6 response after incubation with different concentrations of a 1:1 mixture of STmGMMA and SEnGMMA in comparison to *S. sonnei* 1790-GMMA

A rabbit pyrogenicity study was performed using intramuscular administration of undiluted single GMMA formulations and 2-component iNTS-GMMA vaccine. The 2-component iNTS-GMMA vaccine showed a mean peak temperature rise of 1.1^{O}C using the highest target human iNTS dose. This mean peak temperature rise was 0.8 ^{O}C less than the mean peak temperature rise without Alhydrogel, suggesting whilst there is minimal change in immunogenicity, reactogenicity is reduced with the addition of Alhydrogel.

	Group mean initial temperature [°C]	Group mean maximum temperature [°C] with time indicated	Group mean temperature [°C] at end of measurement [5 hours after vaccination]	Mean peak temperature time point after vaccination [minutes]	Mean peak temperature rise [°C]
iNTS-GMMA (STmGMMA/Alhydrogel + SEnGMMA/Alhydrogel) 20 µg + 20 µg [OAg]	38.6	39.7	39.6	210	1.1
Unformulated mixture of STmGMMA + SEnGMMA 2 µg + 2 µg [OAg]	39.3	41.2	39.8	180	1.9

Table 1.2 STn+SEn with or without Alhydrogel dosed rabbits, showing a mean temperature rise of 1.1 ^{O}C in STm/Sen with Alhydrogel (iNTS Vaccine)

A repeat dose toxicology study in rabbits has been performed using the highest anticipated human iNTS dose at Covance Laboratories (Harrogate, UK).

Group number	Group description	Dose level (µg GMMA [OAg])	Dose Days	Animal numbers			
				Main necropsy		Recovery necropsy	
1	Control (Saline)	0	1, 15, 29, 43	5 Male	5 Female	4** Male	5 Female
2	Test	20 + 20*	1, 15, 29, 43	5 Male	5 Female	5 Male	5 Female
*The test article was 0.5 mL of a bed-side mixing of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel each containing 20 µg of each GMMA (quantified based on OAg) and 0.35 mg Al^{3+} Alhydrogel **One animal was withdrawn during the pre-immunization phase							
Main necropsy: Day 46 (3 days after last injection) Recovery necropsy: Day 71 (28 days after last injection)							

Table 1.3 Outline of repeat dose toxicology study in rabbits. INTS GMMA vaccine versus Saline Control Group

New Zealand White rabbits were grouped into a test and control (saline) group. They then received 0.5ml (40µg OAg) of the iNTS-GMMA vaccine at 2 weekly intervals. The results indicated a systemically well-tolerated iNTS GMMA vaccine. Please see the Investigator's Brochure for further details.

5.12 RATIONALE / AIM OF TRIAL

Thirty to forty-two participants in the 18-55 year age group will be recruited into the trial. The trial will follow a dose escalation design with the first group randomised 1:1 to receive a lower dose of 10.6 µg total OAg of iNTS-GMMA vaccine (5.3 µg of STmGMMA as OAg and 5.3 µg of SEnGMMA as OAg) or placebo. A second group will be randomised 1:1 to receive the full intended dose of 40 µg total OAg of iNTS-GMMA (20 µg of STmGMMA as OAg and 20 µg of SEnGMMA as OAg) or placebo. A third group will be randomised 2:1 to receive the iNTS-GMMA vaccine at intended full dose versus a placebo (See 7.Trial Design). An adequate safety review from the Data Safety Monitoring Committee (DMSC) will allow progression of the trial from group 1 to group 2 and from group 2 to group 3. Given there are no current licensed iNTS vaccines, the comparator for this trial will be an Alhydrogel placebo. The last dose of the vaccine will be on Day 168, with a final visit 6 months thereafter. Thus, participants will remain on the trial for 12 months in total.

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine. The immunogenicity of the vaccine will be assessed by OAg ELISA and serum bactericidal assays specific to each vaccine serovar. Additional exploratory immunological assays assessing functional antibody (including but not limited to opsonophagocytosis and glycosylation), B cell and T cell responses will be performed. If the results of this trial indicate a vaccine with an adequate safety profile, progression to a further phase 1 clinical study in an iNTS endemic country will occur.

6 OBJECTIVES AND OUTCOME MEASURES

6.1 PRIMARY OBJECTIVE

To determine the safety and tolerability between two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.2 SECONDARY OBJECTIVE

To investigate the immunogenicity at two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.3 EXPLORATORY OBJECTIVES

To further investigate the immunogenicity using exploratory immunological analyses of the two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals

6.4 PRIMARY ENDPOINTS / OUTCOME MEASURES

The recording and assessment of local and systemic adverse events following administration of each vaccine dose;

- Tenderness and pain at the injection site
- Induration
- Redness

- Swelling
- Headache
- Malaise
- Myalgia
- Nausea and/or vomiting
- Diarrhoea
- Abdominal Pain
- Anorexia
- Arthralgia
- Fatigue
- Fever
- Blood parameters (haematology / biochemistry)
- Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR

6.5 SECONDARY ENDPOINTS / OUTCOME MEASURES

Immunological assays to study immune responses to vaccines, including:

1. Antibody concentration against serovar specific O antigens determined by enzyme linked immunosorbent assay (ELISA) before and after each dose.

6.6 EXPLORATORY ENDPOINTS / OUTCOME MEASURES

Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to:

1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose .
2. Serum bactericidal antibody (SBA) titres against vaccine homologous strains before and after each dose
3. Serum bactericidal antibody (SBA) titre isogenic strains before and after each dose
4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose
5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose
6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose
7. Transcriptomic profile analysis after immunization to investigate differential expression of innate, B cell and T cell activation gene modules and DNA storage for investigation of the genetic associations with the immune response
8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays

10. Faecal antibody concentration against O antigen determined by by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection.
11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.

7 TRIAL DESIGN

7.1 OVERVIEW OF TRIAL DESIGN

This is a first in human, phase 1, single-centre participant-observer blind study to assess the safety and immunogenicity of three administrations of iNTS-GMMA vaccine in healthy adults. Participants will be considered enrolled in the study once their first vaccination has been administered. The total number of participants is 30-42, and individuals will be divided into 3 groups as described below:

1. **<u>Group 1</u> (PARTICIPANT-OBSERVER BLIND)** - lower dose 10.6 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or a placebo according to the study plan outlined in section 7.2.1. A favourable DSMC review of the safety data from this arm will be required before commencement of group 2. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

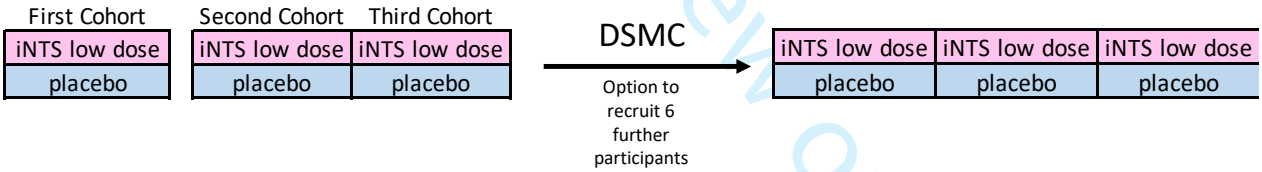


Figure 2.1 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive lower dose iNTS-GMMA vaccine or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

2. **<u>Group 2</u> (PARTICIPANT-OBSERVER BLIND)** - full dose 40 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will only proceed after DSMC review and approval of Group 1. This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 40 µg total OAg of iNTS GMMA vaccine or a placebo. See section 7.2.1 for further details regarding safety and dose escalation. A favourable DSMC review of the safety data from this arm will be required before the commencement of group 3. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

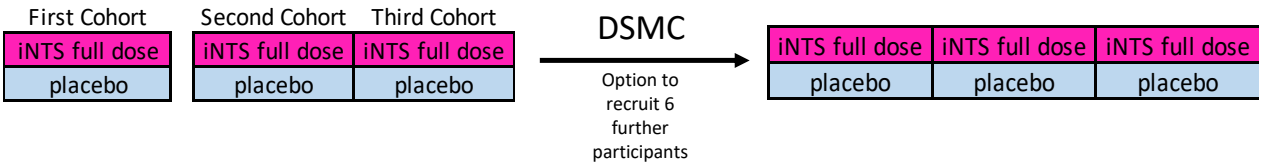


Figure 2.2 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive iNTS full dose or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

3. **Group 3 (PARTICIPANT-OBSERVER BLIND)** – 18 participants will be randomised 2:1 to receive three administrations at D0, D56 and D168 of either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or a placebo. Recruitment to this group will be subject to a favourable interim review of the safety data of group 2 by the DSMC.

   Depending on DSMC review and participant numbers in Group 1 and 2, 18 participants will be enrolled into Group 3:
   Either:

iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.3 Eighteen Participants, randomised 2:1 to receive full dose iNTS-GMMA vaccine or placebo

Or:

iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.4 Eighteen Participants, randomised 2:1 to receive lower dose iNTS-GMMA vaccine or placebo

7.2 DOSE ESCALATION

As this is a first in human trial, we will operate a dose escalation policy in groups 1-3 between lower and full doses. The rationale for these doses is based on experience from animal toxicology studies (5.11 TOXICOLOGY). Escalation between these doses will be dependent on a favourable safety review by the DSMC. This unblinded safety review will consist of AEs from all participants vaccinated in group 1, 2 and 3 at the DSMC review time points indicated below. Group 1 and 2 must have a minimum of 6 participants vaccinated in each group prior to DSMC review. AE Safety Data will include solicited, unsolicited, observation related, laboratory, SAE / SUSARS. Note SAE/SUSARs have specific DSMC reporting instructions as outlined in Section 11.6.

7.2.1 DOSE ESCALATION PROCESS FOLLOWING D0 VACCINE

Group 1 – Lower dose IMP

Initially, 6 participants will be subdivided into 3 pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or placebo. Initially

only one pair will be vaccinated and observed for any adverse reactions for 48 hours before further participants are vaccinated. The medically qualified investigator will be asked to provide the decision on whether to proceed after a blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a second and third pair will be vaccinated at least one hour apart. This will bring the total number of participants in this group to 6, of which 3 receive the lower dose IMP, and 3 receive the placebo.

Group 1: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 1. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same lower dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 2 will proceed.

If participants in Group 1 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 2 – Full dose IMP

In the full dose group, 6 participants will initially be subdivided into 3 pairs as in group 1. Within each pair the two participants will be randomised 1:1 to receive the full dose 40 µg total OAg of iNTS-GMMA vaccine or placebo. Initially only one pair will be vaccinated and observed for any adverse reactions/events for 48 hours before more volunteers are vaccinated. The medically-qualified investigator will be asked to provide the decision on whether to proceed after blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a further two paired cohorts will be vaccinated with the 40 µg total OAg dose. This will bring the total number of participants in this group to 6, of which 3 receive the full dose IMP, and 3 receive the placebo. The second and third pairs will be vaccinated at least one hour apart.

Group 2: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 2. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same full dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 3 will proceed.

If a favourable opinion is given for Group 1, but not for Group 2, then the DSMC may allow the ‘lower dose’ to be used in Group 3 instead of the full dose. If favourable decision is received

from the DSMC a substantial amendment would be required to continue further vaccination clearly stating the iNTS-GMMA vaccine dose to be administered.

If participants in Group 2 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 3 – Low or Full Dose

In this group, 18 participants will be randomised 2:1 to receive the iNTS GMMA vaccine (either the lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo. The total number of participants in the study will be between 30-42 individuals, depending on the final numbers of participants recruited to group 1 and 2.

7.3 DSMC (Unblinded) REVIEWS

The DSMC will review the unblinded safety data at two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

7.4 GROUP ALLOCATION

Allocation to each group will be decided by order of enrolment into the trial. Participants will be considered enrolled in the trial once they receive their first vaccination. Groups 1, then 2 will be preferentially recruited to ensure study progress as per the dose escalation process above with randomisation within each cohort pair. Once safety data have been reviewed by the DSMC, participants recruited to group 3 will be randomised to receive either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo in a 2:1 ratio.

7.5 SAFETY MONITORING

Safety outcomes of the participants will be monitored throughout the study. This will be done by monitoring symptoms at visits, daily review of an electronic symptom diary up to D6 after every vaccine received on the study, and safety blood tests (Table 2.1).

Group 1 and 2 vaccinations will proceed in a staggered fashion (Fig 2.2), dependent on safety review by the DSMC. The time interval between the 7 days post the last participant to receive their first vaccine administration in Group 1 to the first participant in Group 2 to receive their first vaccine administration therefore allows continuous monitoring of the safety of both the second (D56) and third (D168) vaccinations in Group 1 participants receiving low dose IMP prior to the respective vaccinations in Groups 2.

The medically qualified investigator will be asked to provide the decision on whether to proceed to second (D56) and third (D168) vaccine administration in Group 2 after reviewing 7 days of blinded safety data after the last participant receives the second and third vaccine administrations in Group 1 respectively (See section 11.14). This review will consider blinded safety data from

the results of participant e-diaries, eCRFs, AEs after vaccination and safety bloods. This review will be based on the clinical judgement of the investigator with the option to escalate to the DSMC if any concerns. A similar review will be conducted using the safety data from the last participant to receive their second (D56) and third vaccine (D168) administrations of Group 2 prior to proceeding the the second (D56) and third (D168) vaccine administration of Group 3.

Cumulative toxicity has not been observed with similar GMMA based *Shigella* vaccines and is not expected with this vaccine. Thus staggered vaccination of cohorts of second and third vaccines in Group 1 and 2 will not be performed.

Full details about the reporting of any adverse events or serious adverse events, and the role of the DSMC beyond the dose escalation reviews is discussed in section 11 of the protocol.

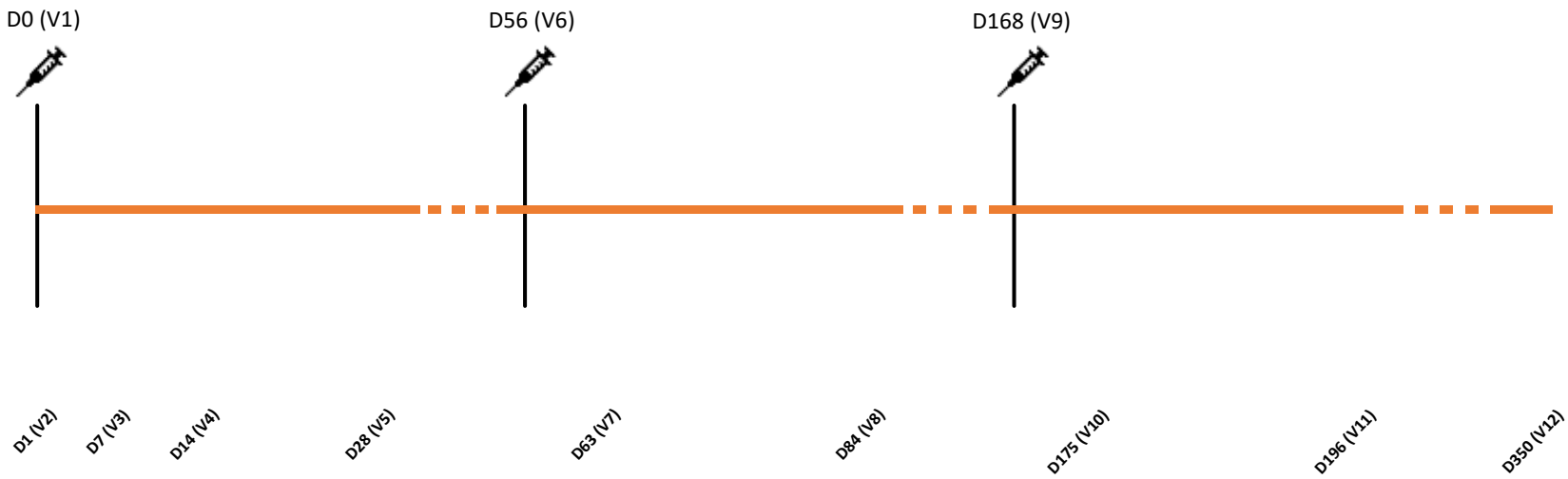
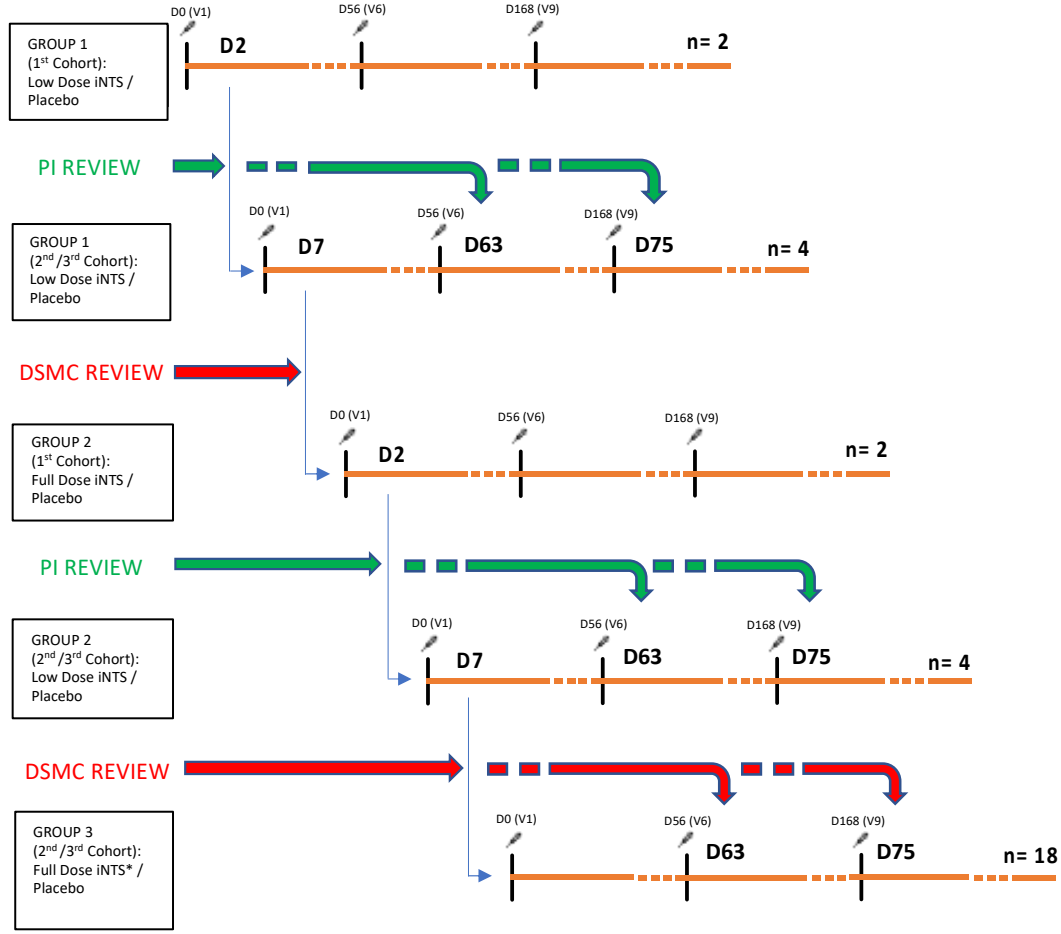


Figure 2.1 Vaccine schedule: Groups 1 – 3



* Low Dose iNTS may be considered if unacceptable safety signal in Group 2 high dose iNTS following Investigator and DSMC review

Figure 2.2 Dose Escalation Schedule: Groups 1 - 3

	Screening	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Indicative Study Day		D0	D1	D7	D14	D28	D56	D63	D84	D168	D175	D196	D350
Day post last vaccine		0	1	7	14	28	56	7	28	112	7	28	182
Visit Window (days)	V1 - 90*		0	+/- 1	+/- 2	+/- 7	+/- 4	+/- 1	+/- 7	+/- 4	+/- 1	+/- 7	+/- 28
Informed consent	x												
Confirmation of eligibilty criteria		x					x			x			
Obtain 24 hr contact details		x					x			x			
Medical history (including demographics and medication)	x												
Interim medical history (including concurrent medication)		x	x	x	x	x	x	x	x	x	x	x	x
Review / collection of AEs and SAEs since last visit		x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x												
Vital signs	x	x	x	x	x	x	x	x	x	x	x	x	x
Urine pregnancy test	x	x					x			x			
Urine dipstick (with urine analysis as needed)	x												
Blood sample	x	x	x	x	x	x	x	x	x	x	x	x	x
Oral Fluid Swab		x			x	x	x	x	x	x	x	x	x
Stool/Faecal Sample (Optional Only)		x										x	x
Vaccination		**x**					**x**			x			
e-Diary entries		x	x	x			x	x		x	x		
Intervention arm allocation		x											

Table 2.1 Visit Structure: Groups 1 - 3

Participants will complete the scheduled visits within the visit windows outlined above, however due to unforeseen circumstances such as participant unavailability, the visit may still proceed outside of window if reasonable to do so, as judged by the investigator. A physical exam is performed at screening. A physical exam is not routinely performed at the remaining visits unless the clinical history or situation deems it necessary.

*Once the screening visit has been completed, V1 must be scheduled within a maximum of 90 days of the date of the screening visit. This interval allows for receipt of GP medical summaries or repeat blood tests. If the V1 for the potential participant falls outside of this period, a repeat screening visit must be performed if the participant remains eligible and willing. However certain tasks which are unnecessary to be repeated as judged by the investigator may be brought forward. Tasks which must be repeated at this re-screening visit include: consent, interim medical history and urine pregnancy test (if applicable). Other tasks may also be performed at the Investigator's discretion.

8 PARTICIPANT IDENTIFICATION

8.1 TRIAL PARTICIPANTS

Male or female participants aged 18-55 years inclusive who are in good health (as determined by a study doctor) and who are able to provide written informed consent, will be eligible for inclusion in this study. Between 30-42 participants are required.

8.2 INCLUSION CRITERIA

Participants must satisfy all of the following criteria to be considered eligible for the study:

- Willing and able to give informed consent for participation in the study
- Aged between 18 and 55 years inclusive
- In good health as determined by
    - Medical history
    - Physical examination
    - Clinical judgment of the investigators
- (Females) Willing to use highly effective contraception as defined in Section 8.5 from one month prior to receiving the first vaccine and for the duration of the study
- Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
- Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study.
- Willing to allow study team access to medical records for the purposes of eligibility assessment and / or safety follow up during the trial.
- Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS).

8.3 EXCLUSION CRITERIA

The participant may not enter the study if any of the following apply:

- History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators. This includes any history of **significant** disease in the following:
    - Cardiovascular disease including congenital heart disease, previous myocardial infarction, valvular heart disease (or history of rheumatic fever), previous bacterial endocarditis, history of cardiac surgery (including pacemaker insertion), personal or family history of cardiomyopathy or sudden adult death
    - Respiratory disease such as uncontrolled asthma and chronic obstructive pulmonary disease
    - Endocrine disorders such as diabetes mellitus and Addison's disease
    - Significant renal or bladder disease
    - Biliary tract disease

    - Gastro-intestinal disease such as inflammatory bowel disease, abdominal surgery within the last two years, coeliac disease and liver disease (including hepatitis B or C infection)
    - Neurological disease such as seizures and myasthenia gravis
    - Haematological disease including coagulation problems
    - Metabolic disease such as glucose-6-phosphate dehydrogenase deficiency
    - Psychiatric illness requiring hospitalisation
    - Depression, anxiety or other psychiatric illness whose severity is deemed clinically significant by the study investigators
    - Known or suspected drug and/or alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week)
    - Non-benign cancer, except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ
- Have any known or suspected impairment or alteration of immune function, resulting from, for example:
    - Congenital or acquired immunodeficiency (including IgA deficiency)
    - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
    - Autoimmune disease
    - Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy (including for more than 7 days consecutively within the previous 3 months).
- Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing (at the discretion of an Investigator) within the recruitment timeline of the study
- Have received any oral typhoid vaccination (e.g. Ty21a or M01ZH09) within the last 3 years or a paratyphoid vaccine (as part of a clinical trial)
- Have participated in previous typhoid or paratyphoid challenge studies (with ingestion of challenge agent).
- Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
- Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (COVID-19 vaccine exempt, see Section 9.14)
- Any history of allergy or anaphylaxis to a previous vaccine or vaccine component
- Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
- Participation in another research study involving an investigational product or that which may compromise the integrity of the study (e.g. significant volumes of blood already taken in previous study) in the past 12 weeks, or are planning to do so within the trial period
- Planned donation of blood/blood products outside of the study and during the trial period.
- Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
- Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study[1]

[1] As defined by CTFG Recommendations related to contraception and pregnancy testing in clinical trials, current document: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf [accessed 23rd March 2022]

- Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m^2
- Any other significant disease or disorder which, in the opinion of the Investigator, may:
  - Put the participants at risk because of participation in the study
  - Influence the result of the study
  - Impair the participant's ability to participate in the study

8.4 TEMPORARY EXCLUSION CRITERIA

The following applies to both **initial enrolment** and **subsequent vaccination** visits. If the temporary exclusion resolves within the time constraints of the trial, they can be enrolled and/or progression in the trial can continue.

- Receipt of any systemic corticosteroid (or equivalent) treatment within 14 days prior to vaccination, or for more than 7 days consecutively within the previous 3 months
- Febrile illness (oral temperature ≥37.5°C) or systemically unwell on the day of vaccination
- If a participant is taking systemic antibiotics then the vaccination is postponed until 7 days after the last dose. This does not apply to topical antibiotic preparations
- Use of antipyretics in the 4 hours prior to vaccination
- A laboratory AE considered, in the opinion of the Investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered
- Symptoms of COVID-19, without confirmation of infection (as per current government guidelines) 14 days prior to vaccination visit
- Validated positive (first of episode) SARS-CoV-2 test (NAAT or antigen) within 4 weeks prior to vaccination visit
- Any illness / AE considered, in the opinion of the investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered

8.5 PREGNANCY AND CONTRACEPTION

The possible adverse effects of the iNTS-GMMA vaccine on the outcome of pregnancy are unknown; therefore, pregnant women will be excluded from the study. Women of childbearing potential will be required to use an effective contraceptive measure. Contraception should be maintained during the vaccination period and for the duration of the study. Should a volunteer become pregnant during the trial, she will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. We would not routinely perform venepuncture in a pregnant volunteer unless there is clinical need.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible. The active components of the iNTS-GMMA vaccine are the GMMA particles which consist of blebs of the outer membrane of the two Salmonella serovars, S.Enteritidis and S.Typhimurium. As a result, both components lack the necessary machinery for replication in vivo. They have been designed to stimulate an immune response to the antigen(s) contained within the GMMAs. Together with the lack of replicative machinery and a maximum iNTS-GMMA dose of 40µg makes the risk of human teratogenicity/fetotoxicity possible/unlikely.

A woman is considered of childbearing potential, i.e fertile, following menarche and until becoming post- menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophrectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient.

Female volunteers of childbearing potential are required to use a highly effective form of contraception until their last follow-up visit. Acceptable forms of contraception for female volunteers include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral/intravaginal/transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral/injectable/implantable)
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised male partner
- sexual abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

9 TRIAL PROCEDURES

9.1 RECRUITMENT

Identification of study participants

In order to recruit the required cohort of 30-42 participants, several strategies may be employed, including but not limited to:

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor's surgeries, tertiary education institutions and other public places with the permission of the owner/ proprietor.
- Direct mail-out / SMS/text message / emails: Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases. These include the National Health Applications and Infrastructure Services (NHAIS) via a NHAIS data extract or equivalent. For the NHS databases initial contact to potential participants will not be made by the study team. Instead study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd (or equivalent company), in order to preserve the confidentiality of potential participants. CFH Docmail Ltd (or equivalent company) is accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70). For mail-outs via the electoral register, we will have access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH

Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company). Volunteers may also be recruited using direct SMS/text message, or emails to potential participants identified by GPs from their databases.

- Email campaign: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and link to study website by email or hard copy.
- Oxford Vaccine Centre (OVC) database for healthy volunteers: Direct email and link to members of the public who have registered their interest in potentially volunteering for clinical trials conducted by OVC. This secure database is maintained by OVC and members of the public registered here have given consent to have their details recorded and be contacted expressly for this purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to volunteering for any trial they are contacted about.
- Media advertising: Local media, newspaper and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- Website advertising: Description of the study and copy of information booklet on the OVG website.
- Social media: Advertisements placed on OVG or University of Oxford Social media accounts or targeted social media platform advertisements including, but not restricted to, Twitter, Facebook and Instagram
- Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

Recruitment, approach and initial eligibility assessment of potential study participants
Potential participants who are interested in the study will be able to contact the OVG by telephone, email or trial website for further information. Once an expression of interest has been received by OVG, an information sheet will be sent via mail, email or downloaded from the website by potential participants to read at their leisure. If participants are willing to proceed, they will be initially screened by a website questionnaire and/or telephone before they are invited for a screening and consent visit, where their eligibility will be assessed by member of the clinical research team at the Oxford Vaccine Group. Permission to access the volunteer medical records either via the electronic records system or GP will be sought (if possible) prior to the screening visit. Participants will be asked to sign a secure electronic document (hosted by the REDCap database) which will then be counter-signed by a study team member. Alternatively written permission to access medical records will be sought at the volunteers screening and consent visit.

9.2 INFORMED CONSENT

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Consent will be sought as described in relevant SOPs.
Written and verbal versions of the participant information booklet and informed consent form will be presented to the participant, detailing no less than:

- the exact nature of and the rationale for performing the study
- implications and constraints of the protocol

- the risks and benefits involved in taking part

It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. The participant will be allowed at least 24 hours to consider the information from when they receive it, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study.

The participant will have the opportunity to discuss the study with a medically qualified investigator. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the appropriately trained and delegated clinician. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site.

Participants will be informed that they would also be eligible for BioBank ('Oxford Vaccine Centre Biobank' Southampton & South West Hampshire LREC (B) 10/H0504/25). BioBank is a separate study and optional to all participants of studies conducted by OVC. Separate consent is sought for this.

9.3 BASELINE ASSESSMENTS AT SCREENING

Once informed written consent is obtained, the following baseline assessments and information is collected as part of the assessment of inclusion/exclusion criteria:

- Participant demographics; age, sex and ethnicity
- Travel history (record travel to any country outside of the UK for longer than 2 months; record any travel to Sub-Saharan Africa or South East Asia)
- Medical history
- Contraception; female participants are asked if they are willing to use effective contraceptive measures one month prior to vaccination and for the remainder of the study
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- Recording of resting pulse, blood pressure, temperature, weight and height
- Physical examination; cardiovascular, respiratory, abdominal and gross neurological examination
- Urine dipstick (and laboratory analysis if appropriate) and urine pregnancy test
- Blood samples for: haemoglobin count, white cell indices, platelet count, serum sodium, serum potassium, serum urea, serum creatinine, liver function tests, C-reactive protein, HIV, Hepatitis B and C.
- Collect emergency contact details

The medical, vaccination and prescribed medication history are initially based on participant recall. However, with prior participant approval, patient medical summary, vaccination and prescribed medication history will be formally requested from the GP or accessed via the electronic patient record (if available) at the screening visit if not already requested or accessed in advance. In addition, all participant GPs will be notified of their participation in the study.

Consent will be taken to register the participant on The Over-volunteering Prevention System (TOPS) database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs and blood donations. This will be done using the

participant's National Insurance number or Passport Number. The TOPS database will be checked for any conflicts at screening, however formal registration will be done at enrolment.

9.4 RANDOMISATION

Groups 1 and 2 will be cohorted in pairs randomised 1:1 to receive the lower dose or full dose iNTS GMMA vaccine respectively versus placebo. Once the study has progressed to Group 3, 18 participants will be randomised 2:1 to receive the investigational product versus placebo.

Randomisation will be conducted using an electronic system within the RedCAP database. Participants in Group 1 and 2 will be randomised 1:1 in blocks of two, to meet the total numbers required for each group. Participants in Group 3 will be randomised 2:1 in blocks of three, to meet the total numbers required.

9.5 BLINDING AND CODE-BREAKING

This study will be conducted in an observer and participant blind fashion. The study blind will be maintained from the time of participant randomisation until participant unblinding which will occur once the last participant has completed their final visit.

There will be dedicated blinded and unblinded study teams. Blinded staff will include clinical study doctors (including the CI), study nurses, administrative and laboratory staff who will be directly managing participants and participant samples. Vaccine and placebo will be reconstituted, checked and administered by a dedicated unblinded study team, such that the participant will not be aware of which vaccine they have received. Assays requiring blinding in the laboratory (eg Elispots), will be measured by at least one individual blinded to vaccine or placebo allocation.

Participants and their General Practioners will receive written notification by letter or email of whether they have received the vaccine or placebo at the time of full study unblinding.

Unblinding may also occur at an earlier time point in the event of the occurrence of SAEs, SARs or SUSARs (please see section 11).

In the case of medical emergency the investigator will have direct access to unblinding of the participant(s) by opening of the participant(s) sealed envelope containing their vaccine record. This will be confirmed by electronic unblinding via the REDCap database, which can be performed in the first instance to avoid any delay in unblinding due to a medical emergency or out of hours.

This will be conducted under the guidance of the Data Safety and Monitoring Committee. Unblinding procedures will be conducted in accordance with local OVG SOPs.

9.6 VACCINATION VISITS

Vaccination visits are held at the CCVTM. The visit procedure for the vaccination visits will be as follows:

- Ensure that participant consent remains valid and confirm continued consent.

- Measure Weight, Height and Body Mass Index
- Obtain and document interim medical history since the last visit and check eligibility criteria, specifically temporary exclusion to vaccination
- Review for AEs and SAEs since the last visit
- Training on electronic diary card entry and if second or third vaccine review of diary card entries and laboratory AE profile
- Record oral temperature, pulse and blood pressure
- Perform urinary pregnancy test for females
- Perform blood draw
- Perform oral fluid swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Administer vaccine by IM injection into non-dominant deltoid muscle by the second team member in second clinic room (who remains unblinded, following iNTS-GMMA vaccine preparation as per clinical study plan)
- Observe for immediate adverse events for 60 minutes, followed by post vaccine checks including routine observations, review of vaccine site and assessment of wellbeing
- Schedule next visit and re-iterate participant requirements such as return of the Diary Card entries

9.7 NON-VACCINATION VISITS

Other visits may require the following procedures:

- Obtain and document interim medical history since screening and check continued eligibility
- Review for AEs and SAEs since the last visit
- Review eDiary entries and laboratory blood tests
- Record oral temperature, pulse and blood pressure
- Perform blood draw
- Perform oral swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Schedule next visit and re-iterate participant requirements such as eDiary entries

9.8 OUTSIDE OF CCVTM VISITS

Participants will be asked to maintain a diary card describing all (solicited and unsolicited) adverse events up to seven days post vaccination. If there is an ongoing adverse event recorded in the diary the participant will be asked to continue with ediary entries until resolution depending on the nature of the adverse event and feasibility to do so as judged by the clinical investigator. Laboratory results are also entered into a safety results database in real-time or on demand for active monitoring throughout the study by a member of the study team. Each participant will be able to access a member of the study team 24-hours per day via a study-specific emergency number should they have any concerns or are in need of advice.

9.9 LABORATORY INVESTIGATIONS

In addition to blood samples needed for the safe conduct of the trial and assessment of the primary endpoint, blood/oral fluid/stool samples from the participants will also be subjected to laboratory analyses in order to assess the objectives defined in the secondary and exploratory endpoints. The plan for analysis is outlined below, and will be further detailed in a specific analysis plan:

9.9.1 ANALYSIS OF BACTERICIDAL ACTIVITY (GVGH)

The ability of the antibodies in participants serum samples to mediate killing of *S.* Typhimurium and *S.* Enteritidis in the presence of complement (serum bactericidal activity (SBA), will be quantified. The target strains in the SBA assay will be one wild-type strain per serotype in order to elucidate the antigen-specific SBA using a high-throughput luminescence assay developed by GVGH.

9.9.2 FURTHER ANALYSIS OF FUNCTIONAL ANTIBODIES (OVG and Collaborators)

The ability of the antibodies in participants' serum samples to mediate killing of a panel of *Salmonella* bacteria to assess cross protection of the iNTS-GMMA Vaccine. Further exploratory antibody analyses including but not limited to SBAs, opsonophagocytic assay and Fc glycosylation may be performed by OVG and collaborators.

9.9.3 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST O-ANTIGENS (GVGH)

Serum IgG antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. Test samples will be analysed at three dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Anti-OAg responses will be expressed in ELISA units. Plate coating antigens are well characterized OAg purified by GVGH.

9.9.4 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST PORIN AND OTHER ANTIGENS (OVG and Collaborators)

Antibody responses against other antigens from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point may be analysed by ELISA developed and performed by OVG and collaborators. Test samples may be analysed at multiple dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Antigen responses will be expressed in ELISA Units.

9.9.5 CELLULAR RESPONSES AND CYTOKINE RELEASE (OVG and Collaborators)

Laboratory analyses to quantify the B-cell and T-cell responses specific to STmGMMA and SEnGMMA components of the vaccine will be performed when feasible using peripheral blood mononuclear cells (PBMCs) derived from study participants sampled before, and at several time points after each dose, using the assays described below.

9.9.6 ANALYSES OF B CELL RESPONSES (OVG and Collaborators)

The ability of the STmGMMA, SEnGMMA and the iNTS-GMMA vaccine to stimulate a detectable increase in antigen-specific memory B cells and plasma cells will be enumerated by ELISPOT using plates coated with vaccine antigens (such as individual GMMA or their

respective purified O antigen) or B cell mitogens. The phenotype and kinetics of the B-cell subsets involved in the response will be determined using fluorescent-labelled antibodies in a flow cytometric assay. In addition, other assays to monitor the B-cell immune response to the vaccines may be performed if sufficient samples are available.

9.9.7 ANALYSES OF T CELL RESPONSE AND CYTOKINE RELEASE (OVG and Collaborators)

In order to evaluate the ability of the iNTS-GMMA vaccine to stimulate T cell responses, we aim to quantify when possible vaccine-induced responding T-cells by multicolour flow cytometry and mass cytometry. Effector T cells will be clones and rested for antigen specificity against iNTS bacterial strains and tested for their capacity to recognise proteins contained within individual GMMAs. Moreover, other assays to monitor the T-cell immune response and cytokine release to the vaccines will be performed if sufficient samples are available.

9.9.8 ANALYSIS OF GENE EXPRESSION (OVG and Collaborators)

RNA will be extracted from a small volume of peripheral blood (~1ml) at three study visits (as per Table 3.1) for analysis of gene expression profiles. This analysis will be used to highlight differences in gene expression induced by vaccination and provide insight into the immunobiology of vaccine responses.

9.9.9 ANALYSIS OF GENETIC DETERMINANTS OF VACCINE RESPONSE (OVG and Collaborators)

DNA samples obtained, from peripheral blood, will contribute to a Biobank of samples from multiple different Oxford Vaccine Group studies. These DNA samples will be used to analyse the genetic factors influencing vaccine responses (immunogenicity and reactogenicity). DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses. This specific goal will therefore not contribute to the results of this individual study.

9.9.10 ANALYSIS OF ORAL FLUID ANTIBODY CONCENTRATION AGAINST O ANTIGENS AND PORINS (OVG and Collaborators)

Oral fluid samples will be collected via an oral fluid swab as detailed in the Clinical Study Plan. Antibody responses against OAg and porin Ag from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. OAg and Porin Ag responses will be expressed in Antibody Units.

9.9.11 COLLECTION OF SERUM TO BE USED AS A REFERENCE STANDARD FOR THE SET-UP OF LABORATORY ASSAYS IN CURRENT / FUTURE STUDIES (OVG/GVGH and Collaborators)

Serum samples (30mls) will be collected at Visit 8 (28 days post 2nd Vaccine) from recipients of the iNTS-GMMA vaccine within group 3. Serum will be processed as per OVG SOPs and stored at -80°C prior to transfer to GVGH for the development of the serum standard. Serum IgG responses to *S*. Typhimurium and *S*. Enteritidis will be screened by O Antigen ELISA. Samples

will be selected based on ELISA optical density (OD), characterised, calibrated and pooled. The calibrated human serum pool (or serum standard) will allow development of an ELISA standard curve by which test samples may be compared for quantification of antibody titres.

9.9.12 ANALYSIS OF FAECAL ANTIBODY CONCENTRATION AGAINST O ANTIGENS (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing a stool/faeces sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOP. Antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis will be analysed by ELISA. OAg responses will be expressed in Antibody Units.

9.9.13 INVESTIGATION OF IMPACT OF VACCINATION ON GUT MICROBIOTA (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing said sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOPs for bacterial whole genome sequencing and 16S RNA sequencing.

9.10 SAFETY BLOOD TESTS

All other laboratory tests including FBC, WBC differential counts, C-reactive protein, urea, creatinine, electrolytes, aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, will be performed using the OUHFT, NHS laboratories. Blood samples will be collected in assay sample tubes and delivered to OUH clinical laboratories for analysis according to national SOPs.

Samples collected as part of this study may also be used for other exploratory studies of scientific relevance by the OVG laboratory or any of the collaborating laboratories which may include the transfer of samples within and outside the EU. These samples may include oral fluid, serum, extracted DNA and RNA, and PBMCs. Frozen samples will be stored under the ethical approval for this study until study completion. At this time, samples will be transferred to the Oxford Vaccine Centre Biobank subject to participant consent (see Section 9.2). Studies may include further investigation of the inflammatory and immunological response to vaccination.

9.11 TRIAL PROCEDURE SCHEDULE

Details of which assays are performed on each visit are recorded in the table below. The total volume of blood obtained per patient over the course of the study will be 637.5 – 667.5mls.

Visits	Days	Vaccine / Post Vaccine Days	Antibody Assay: SBA, OAg (GSK)	Antibody / Porin Assays (OVG)	PBMCs (T Cell assays)	PBMCs (B Cell assays)	Functional Antibody Assays / Extracted from PBMCs	Serum Standard*	Transcriptomics	FBC	U&E, LFT, CRP	Viral Serology	Oral OAg/Porin IgG/A Assays	Stool/Faecal Ig Assays/Microbiota	Total (mls)
Screen	-	-	-	-	-	-	-	-	-	1	2	4	-	-	7
V1	D0	Vaccine 1	10	5	50	-	+	-	2.5	1	2	-	+	+	70.5
V2	D1	+1	-	-	-	20	-	-	2.5	-	-	-	-	-	22.5
V3	D7	+7	10	-	24	10	-	-	2.5	1	2	-	-	-	49.5
V4	D14	+14	-	5	24		-	-	-	-	-	-	+	-	29
V5	D28	+28	10	5	24	20	+	-	-	1	2	-	+	-	62
V6	D56	Vaccine 2	10	5	24	20	+	-	-	1	2	-	+	-	62
V7	D63	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V8	D84	+28	10	5	24	20	+	30*	-	1	2	-	+	-	62 (92*)
V9	D168	Vaccine 3	10	5	24	20	+	-	-	1	2	-	+	-	62
V10	D175	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V11	D196	+28	10	5	24	10	+	-	-	1	2	-	+	+	58
V12	D350	+182	10	5	24	10	+	-	-	-	-	-	+	+	49
															637.5 (667.5*)

Table 3.1: Blood Sampling (Including Oral Swab) Schedule (in mls)
***Serum standard (30mls) will be collected from participants in Group 3 only**
** Stool Sample collection will be participant opt-in only

Sampling time points, volumes and investigations may vary at the discretion of the PI. Samples may be omitted as per the investigating team's discretion for example if participants develop low haemoglobin as defined and managed as per the SALVO clinical study plan.

9.12 EARLY DISCONTINUATION / WITHDRAWAL OF PARTICIPANTS

Each participant can exercise their right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for participant safety including, though not exclusive to, the following:

- Significant non-compliance with study requirements
- Consent withdrawn
- Lost to follow up

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. The reason for withdrawal, if given, will be recorded in the CRF. A participant who is withdrawn from the study can be replaced if the individual has not received any vaccine. Therefore, lost to follow up subjects who have received at least one dose of vaccine will not be replaced. Furthermore, in circumstances pertaining to the safety of the participant, the

investigator may choose to discontinue further vaccination +/- study procedures, however with ongoing consent may continue to monitor for safety via either scheduled or unscheduled visits. Such circumstances may include though not exclusive to the following:

- Pregnancy
- An adverse event which requires discontinuation of the study vaccinations or results in an inability to continue to comply with study procedures
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. All data and participant samples obtained up to the point of withdrawal will be used in the analysis.

9.13 DEFINITION OF END OF TRIAL

The end-of-study is completion of the last laboratory assay on the last participant sample obtained at Visit 12. End of study must be achieved no later than 8 months after obtaining the last participant last sample at Visit 12.

9.14 SPECIAL CIRCUMSTANCES: COVID-19

9.14.1 STUDY CONDUCT / RISK ASSESSMENT

It is difficult to predict the time course of the COVID-19 pandemic. At all times the safety and welfare of study participants remains paramount.

The Chief Investigator will perform a risk assessment as necessary with relevant parties (e.g. DSMC, Regulatory Authorities or GVGH) on the basis of the current UK COVID-19 situation, to determine:

1. Appropriateness to initiate vaccinations
2. Appropriateness to continue the trial once started
3. Necessity to extend trial duration

Dependant on the prevailing COVID-19 situation, the conduct of the trial may be modified in line with national policy in effect at the time in the interests of participant safety. Such measures may include but are not limited to:

- Pausing further vaccinations
- Modifying study visits and procedures (as detailed in the SALVO Clinical Study Plan) eg: study visits conducted by phone or video calling where appropriate.

Any deviation not outlined in this protocol required due national policy in effect at the time, will require a non-substantial/substantial amendment unless specifically permitted to do otherwise by the Sponsor, MHRA and REC.

9.14.2 COVID-19 INFECTION CONTROL MEASURES AT VISITS

The CCVTM was one of the sentinel sites used by the University of Oxford to implement COVID-19 infection control policies and act as a model for securing a workplace. Further details of infection control procedures including the safe handling of clinic visits, a COVID-19 secure workplace, maintaining staff safety are included in the clinical study plan and OVG SOPs. These will be regularly updated in line with University of Oxford and NHS/UK Government policies.

9.14.3 PARTICIPANTS UNDER QUARANTINE

Given the evolving epidemiological situation both globally and in the UK, should a participant be under quarantine and unable to attend any of the scheduled visits, a telephone/video consultation may be arranged in order to obtain safety and the visit may be re-scheduled, depending on the timelines.

9.14.4 PARTICIPANTS WITH COVID-19 SYMPTOMS

Participants who become symptomatic during follow-up will be instructed to call the study team who will then advise on how to proceed with clinical testing for COVID-19, through the community testing programme, if necessary, as per the Clinical Study Plan. Participants would be expected to report a transient, flu-like illness within 24hours of vaccination. If this reaction should include a fever, we would expect this to resolve within 48hours. If a fever starts and resolves within 48hours of vaccination it will be attributed to the vaccine. If a fever persists for more than 48hours, or starts more than 48hours after vaccination, it will be considered unlikely to be related to vaccination and the participant may be advised to proceed with clinical testing for COVID-19 outside of the study if appropriate.

Participants who develop COVID-19 symptoms and have a positive SARS-CoV-2 test (appropriately validated NAAT or antigen test) after the first vaccination can only receive a subsequent vaccination after a minimum 4 weeks interval from their first positive test of that episode, provided they have had only a mild illness and have fully recovered. Moderate-Severe illness will be defined as 4 or above as per the WHO Clinical Progression Scale (See Appendix D: WHO Clinical Progression Scale for clinical studies of COVID-19). Those who have had moderate-severe disease will not receive further IMP.

In cases of mild or asymptomatic disease, the decision to proceed with booster vaccinations will be at clinical discretion of the investigators, and each case will be evaluated by a study doctor before proceeding (including physical examination and peripheral oxygen saturation recording [Sp02]). The trial clinician must assess that the participant has fully recovered from their illness. Participants must have no ongoing symptoms that could be attributable to their COVID-19 illness and feel that they have fully recovered and are well. For participants who are asymptomatic and have a positive SARS-CoV-2 test (and who remain asymptomatic), a minimum of 4 weeks from positivity will be required before further vaccinations are administered. They will also undergo a physical examination and peripheral oxygen saturation recording before proceeding with IMP.

All relevant details and any required modification in study visits/procedures as per protocol will be documented in a separate adverse event eCRF. Management of these participants will be as detailed above and in the SALVO Clinical Study Plan. Participant follow up and safety reporting will be as detailed in Section 11 Safety Reporting.

9.14.5 PARTICIPANTS INVITED FOR COVID-19 VACCINATION DURING THE TRIAL

Participants who are yet to receive a COVID-19 vaccine but become eligible for this as per UK policy, would be invited to discuss this with the study team.. If agreed by the participant and if it were possible, we would find a mutually agreeable time to receive a COVID-19 vaccine in concert with study timelines and as per exclusion / inclusion criteria (section 8.3). Participants would not be impeded in taking up an offer of a COVID-19 vaccine if offered through the national rollout. If a rollout offer coincides with a planned trial vaccine, the trial vaccine would be rescheduled to 2 weeks before or after the COVID-19 vaccine (whichever was closest to trial schedules). This is in line with the UK Green Book COVID-19 vaccination recommendations for other vaccines which recommends "at least 7 days" interval. It would also minimise the risk of any cross-attribution of reactogenicity, and minimise impact on immunology investigations. If a participant receives a COVID-19 vaccine during the trial, both the vaccine and date of administration will be requested and recorded in the eCRF.

10 TRIAL INTERVENTIONS

10.1 INVESTIGATIONAL MEDICINAL PRODUCT (IMP) DESCRIPTION

10.1.1 iNTS GMMA VACCINE

The vaccine product iNTS-GMMA consists of 2 components: 80 µg OAg/mL STmGMMA and 80 µg OAg/mL SEnGMMA each asceptically formulated independently on Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5. Prior to administration, equal volumes of each component are mixed as specified in the clinical study plan to yield:

- Full dose: 20 µg OAg of STmGMMA/Alhydrogel + 20 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL.
- Lower dose: 5.3 µg OAg of STmGMMA/Alhydrogel + 5.3 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL (3.8 dilution of iNTS-GMMA vaccine full dose diluted with placebo).

10.1.2 PLACEBO

The placebo consists of 0.5 mL Alhydrogel in isotonic 20mM Phosphate buffered saline pH6.5. The placebo is also used for vaccine dilution.

10.2 BLINDING OF IMPS

Group 1-3 will operate a participant-observer blind i.e. both the participant and the observer will be unaware of the group allocation. There will be a trained unblinded team who will prepare and administer the iNTS and placebo vaccine. Both the iNTS and placebo vaccine will appear similar at the point of administration to the participant.

10.3 STORAGE OF IMP

The vaccine product requires storage at 2 to 8oC throughout and vaccines will be transported to the OVG after authorised release for use in the clinical trial by the GSK Qualified Person (QP)

and study approval by EC and MHRA. All movements of study medication between GVGH and OVG, will be documented in accordance with relevant SOPs.

The study treatment will be stored at the OVG in temperature monitored refrigerators with an auditable temperature record in accordance with the manufacturer's instructions and relevant SOPs. Study fridges are connected to a monitoring system with 24-hour access to staff that are able to move the product in the event of significant temperature deviation, for example fridge malfunction as per OVG 001: Vaccine Receipt, Storage, Cold Chain Maintenance and Return/Deposal.

10.4 COMPLIANCE WITH TRIAL TREATMENT

The study investigational product and placebo will be administered by trained unblinded study personnel and will be documented according to GCP guidelines and relevant SOPs. Issues related to compliance are therefore the responsibility of study personnel who have received appropriate training.

Access to the randomisation and vaccination eCRF of the study database will be password protected and restricted to the unblinded study team . In the unlikely event that accidental unblinding (i.e for reasons not outlined in this protocol) of any blinded study team member occurs, this would be recorded as a protocol deviation, and the study team member will be quarantined from taking part in further blinded study activities, in so far as the study progress is not compromised.

10.5 ACCOUNTBILITY OF THE TRIAL TREATMENT

iNTS-GMMA vaccines, placebo (also used as diluent) and empty vials will be manufactured, packaged, labelled and supplied by GVGH. All vaccines (vials and boxes) are labelled with a label specifying 'for clinical trial use only' and no less than the following:

- The clinical trial identifier (by reference code)
- The content of each vial
- Batch and serial number
- Chief Investigator
- Research site

The vaccine will be delivered and stored at the CCVTM pending authorised release for use in the clinical trial.

10.6 CONCOMITANT MEDICATION

The use of all concomitant medication prescribed or over-the-counter, will be recorded in the CRF. There is no restriction on the use of concomitant medication but the use of some prescribed medicines, such as immune suppressive agents, may result in the withdrawal of the participant at the discretion of the Investigator, while others, such as antibiotics, may result in a temporary exclusion.

10.7 EMERGENCY MEDICATION AND PROCEDURES

Participants are required to wait and be observed for one hour after the administration of each vaccine dose for signs of anaphylaxis. All clinical staff are trained and can provide evidence of competency in the acute management of anaphylaxis reactions including the use of intra-muscular adrenaline. This is detailed in relevant SOPs and adrenaline is available at all times of vaccine administration and subsequent observation.

The nearest Accident and Emergency Department is at the Oxford University Hospitals NHS Foundation Trust, which is within minutes by ambulance transfer.

10.8 POST-TRIAL TREATMENT

Study medication will not be continued beyond the trial period.

10.9 OTHER TREATMENTS (NON-IMPS)

No other treatments other than those specified in the protocol above will be administered to trial participants.

10.10 OTHER INTERVENTIONS

No other interventions other than those specified in the protocol above will be administered to trial participants.

11 SAFETY REPORTING

11.1 SAFETY REPORTING DEFINITIONS

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

11.2 CAUSALITY ASSESSMENT

The relationship of each adverse event to the trial vaccine(s) or study procedures must be determined by a CI-delegated blinded clinician / investigator. The relationship of the adverse event with the study procedures will be categorized as not related, possibly related, probably related or definitely related. The delegated clinician will use clinical judgement to determine the relationship using the following definitions:

Not related	• No temporal relationship to vaccine administration ***and*** • Alternative aetiology (clinical, environmental or other intervention), ***and*** • Does not follow pattern of recognised response to vaccine administration	
Related	Possible	• Reasonable temporal relationship to vaccine administration, ***or*** • Event not readily explained by alternative aetiology (clinical, environmental or other interventions), ***or***

		• Similar pattern of response to that seen to vaccine administration.
	Probable	• Reasonable temporal relationship to vaccine administration, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***or*** • Known pattern of response with vaccine administration.
	Definite	• Reasonable temporal relationship to vaccine administration or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***and*** • Known pattern of response to vaccine administration.

11.3 SEVERITY ASSESSMENT

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on the criteria listed in the definition of an SAE in section 11.1 above. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Severity will be assessed by clinical symptoms, signs, diagnosis, laboratory results and observations as per the appendices A, B and C. Use of the appendices will be detailed in the following sections.

11.4 PROCEDURES FOR COLLECTING AND RECORDING ADVERSE EVENTS

Abnormal clinical findings from medical history, examination or blood tests, will be assessed by a blinded delegated clinician / investigator as to their clinical significance using the severity grading criteria for Adverse Events tables (see Appendix A, B, C).

All AEs that are observed by the investigator or reported by the participant irrespective of their relatedness to the study medication will be recorded from the day of vaccination and until 28 days after each vaccination These will be recorded in either the e-diary for the first 7 days after each vaccine, the laboratory safety database or the eCRF. Outside of this window (i.e. from 28 days after each vaccination and until the point of a subsequent vaccination or until the final visit if vaccination course completed), non-serious AEs will only be recorded if they require medical attention (contact with GP, visit to emergency department). These will be recorded in the eCRF. All AEs will be collected and recorded by the blinded study team.

It will be left to the blinded investigator clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from further vaccination / study. Such

judgement may require the unblinding of the investigator +/- the participant as per study procedures.

A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. In such an event, Section 9.10 (Early Discontinuation/Withdrawal of Participants) will apply. All AEs that result in a participant's withdrawal from the study will be, subject to participant consent, followed up where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned. This will involve an end of study assessment at which the requirement for further appropriate care under medical supervision will be determined. If required the participant will be referred to their GP for ongoing medical supervision, until symptoms cease or the condition is deemed resolved or stable.

11.4.1 E-DIARY AEs

Solicited adverse events

Solicited AEs are those listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- Solicited adverse events will be recorded by the participant in an electronic diary and graded by the participant alone (appendix A) from the time of each vaccine administration for 7 days post-vaccination (day of vaccination and six subsequent days).

Solicited adverse events will be reviewed daily by the clinical study team. These are participant-entered. If further action is required including face-to-face medical review, and/or prescribed medication this will be recorded by the study team in the eCRF. Causality will be assigned by blinded CI-delegated clinician / investigator. Any solicited AE which meets the definition of a SAE will be managed and reported as per Section 11.6.

Unsolicited adverse events

Unsolicted AEs are those that are NOT listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- These may be recorded by the participant in an electronic diary from the time of each vaccine administration for 7 days post vaccination.

Unsolicited adverse events will be reviewed at clinic visits. If clarification of any event is required then the study nurse or doctor will seek this from the participant during a clinical visit or by telephone call. These unsolicited adverse events will be recorded in the AE section of the eCRF. Unsolicited adverse events recorded in the e-diary will be severity graded by the participant. Causality will also be assigned by the CI-delegated clinician / investigator as per section 11.2.

Additionally participants will be asked about the occurrence of AEs during visits and if any are elicited (within the period for which the eDiary is open) that have not already been recorded they will be recorded in the eDiary as above.

11.4.2 OBSERVATION RELATED AEs

Physical observations (e.g. temperature, blood pressure) of the patient will be taken at each visit (Section 9.6, 9.7). These will be recorded in the eCRF. If abnormal, a severity grading will be automatically assigned by the blinded study team as per Appendix B.

11.4.3 VISIT ELICITED AEs

Participants will be asked about the occurrence of AEs and if any elicited they will be recorded in the eCRF and graded as per Appendix A and B by a blinded CI-delegated clinician / investigator. Any AEs reported outside of the visits will be recorded in the eCRF, except for AEs elicited during the opening period of the eDiary (see Section 11.4.1).

11.4.4 LABORATORY AEs

During the trial, laboratory results will be entered into a password protected database containing all the trial safety blood results. Severity grading for laboratory AEs is defined in Appendix C. Changes in laboratory values will be recorded as AEs if they are of Grade 2 severity or above. Changes of laboratory values of Grade 1 severity may be recorded as AEs if they are judged to be clinically significant by a blinded CI-delegated clinician / investigator.

If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and advised with regards appropriate medical care. Laboratory results may be out of normal range for a number of reasons (eg hot weather, delayed transit to processing laboratory). Changes in laboratory values will be recorded as AEs if they fall out of prespecified ranges and judged to be clinically significant by a blinded CI-delegated clinician / investigator, if some action (eg repeat testing for likely clinically significant test result, or reduction in blood volume required during blood draws) or intervention is required. There may be certain circumstances where it may be necessary to unblind the participant. Should this be necessary the same procedures will be followed as for unblinding participants for SAEs.
If abnormal laboratory values are the result of pathology for which there is an overall diagnosis then this diagnosis should be reported as one AE only.
A Grade 4 laboratory AE will be considered a SAE.

11.4.5 NOTES ON RECORDING AEs

Pre-existing medical conditions (present prior to enrolment into the study) are considered “concurrent medical conditions” and should not be recorded as AEs. However, if the participant experiences a worsening or complication of the condition, the worsening or complication should be recorded as an AE. Study staff will ensure that the AE term recorded captures the change in the condition (e.g., “worsening of”).

Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect at which point it would fall within the definition of "serious" and the congenital abnormality of birth defect would be reported as an SAE. Pregnancy notification and follow-up reports on pregnancy outcome will be provided to the DSMC with ongoing consent of the participant.

11.4.6 FOLLOWING UP OF AEs

AEs considered related to the active vaccine or placebo will be followed until resolution, the event is considered stable or until non-study causality is assigned. At the end of the study all other ongoing/open AEs will be assessed by a blinded CI-delegated clinician / investigator, to ensure if not already done so, adequate medical follow-up (if required) has been arranged, eg. referral to participant's general practitioner.

11.5 REPORTING PROCEDURES FOR SERIOUS ADVERSE EVENTS

SAEs will be collected throughout the entire trial period (from first vaccination to D350 or withdrawal).

All SAEs must be recorded on a SAE form (paper or electronic) with causality assessed by the blinded investigator and reported by email to the CI. All SAE will be reported to the DSMC Chair (or nominated designee) within 24 hours of discovery or notification of the event. If the SAE is deemed related, the CI will unblind to confirm whether the participant has received study vaccine or placebo. If the CI deems that this is a SUSAR, this will be reported according to the SUSAR reporting procedures below. In the absence of the CI these tasks may be performed by a Co-Investigator.

Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form emailed to the CI and DSMC Chair (or nominated designee).

The chair of the DSMC (or nominated designee) will perform an independent review of SAEs and request any further information required in a manner adherent to the procedures and timelines of the DSMC Charter. Documentation of this review will be kept in the TMF. The DSMC will provide independent real-time safety assessment throughout the study as described below.

11.6 EXPECTEDNESS

Expectedness will be determined according to the reference safety information section of the Investigators' Brochure for iNTS-GMMA vaccine. No IMP related SAEs are expected in this study. All SAEs at least possibly related to iNTS-GMMA vaccine will be considered unexpected and be reported to the MHRA and REC as SUSARs within the regulatory timelines, as in section 11.8.

11.7 SUSAR REPORTING

All SUSARs will be reported to the Sponsor, relevant Research Ethics Committee, GVGH, and to the MHRA. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. Any additional relevant information should be sent within eight days of the report.

The CI or Co-Investigator will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

11.8 FORSEEABLE ADVERSE REACTIONS

The foreseeable ARs following vaccination with iNTS-GMMA vaccine or placebo include, locally; injection site pain/ tenderness, redness, swelling, induration; and systemically, headache, malaise, fever, nausea, vomiting, abdominal pain, anorexia, myalgia, arthralgia, and fatigue.

11.9 DEVELOPMENT SAFETY UPDATE REPORTS

In addition to the expedited reporting above, the CI or CI-delegated study team member shall submit once a year throughout the clinical trial or on request, a Development Safety Update Report (DSUR) to the;

- MHRA
- Research Ethics Committee
- Sponsor (RGEA)
- GVGH

11.10 SAFETY PROFILE REVIEW

The safety profile will be reviewed on a day to day basis by the investigators using a blinded electronic diary, adverse events CRF and safety bloods to date. Any concerns will be referred to the blinded CI. If the CI remains concerned they may consider unblinding and/or escalation to the unblinded DSMC as required.

11.11 TRIAL MANAGEMENT GROUP

The OVG study investigators will form the trial management group (TMG) and will provide on-going management of the trial.

11.12 DATA SAFETY MONITORING COMMITTEE (DSMC)

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. Specifically, data review will be done as follows:

1. Formal review of the safety profile after 7 days of safety data has been collected from group 1 before progression to group 2, and review of group 2 after 7 days of safety data has been collected before progression to group 3, as described in section 7.
2. Formal review of the safety profile after two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the

second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

3. Independent review following any SAE deemed to be related to the trial active vaccine or placebo.
4. Unscheduled reviews on request of the study management committee at a demand and frequency determined by the severity of reported adverse events.

From these reviews the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. A summary of all blinded and unblinded AEs and SAEs to date will be provided to the DSMC on request.

The outcome of each DSMC review will be communicated directly to the TMG and documentation of all reviews will be kept in the TMF. The CI will inform GVGH of the outcome of the DSMC review.

The Chair of the DSMC will also be contacted for advice where the Chief Investigator feels independent advice or review is required.

11.13 OTHER SAFETY REVIEWS

In addition to formal DSMC review, there will be local blinded safety monitoring reviews. As described in section 7.2 and 7.5, there will be formal local blinded reviews of safety data by the CI or CI-delegated clinician / investigator at:

1. Day 2 following vaccination of the first paired cohort in groups 1 and 2, to decide on progression to vaccination of respective second and third paired cohort.
2. Following 7 days of safety data after the last participant to receive their second vaccine in group 1, to decide on progression to administer second vaccines in group 2.
3. Following 7 days of safety data after the last participant to receive their second vaccine in group 2, to decide on progression to administer second vaccines in group 3.
4. Following 7 days of safety data after the last participant to receive their third vaccine in group 1, to decide on progression to administer third vaccines in group 2.
5. Following 7 days of safety data after the last participant to receive their third vaccine in group 2, to decide on progression to administer third vaccine in group 3.

11.14 GROUP HOLDING RULES

Group holding rules are as follows:

SAE

- All Grade 3 adverse events in any individual which is possibly, probably or definitely related to vaccination (i.e. a AR) will be assessed by the CI-designated clinician / investigator to determine whether the event meets the criteria for a SAE related to vaccination (i.e. a SAR) as per Section 11.1. If this criteria is met the this would trigger the following group holding rule.
- An SAE which occurs in any one individual which is possibly, probably or definitely related to vaccination (i.e. a SAR) would trigger a group holding rule.

Solicited/unsolicited/laboratory adverse events

Solicited local adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 solicited local adverse event within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs

Solicited systemic adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any groups are followed by a Grade 3 solicited systemic adverse event beginning within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs.

Unsolicited adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 unsolicited adverse event within 7 days after vaccination (day of vaccination and six subsequent day) and persisting at Grade 3 for >48 hrs.

Laboratory adverse event:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 laboratory adverse event beginning within 3 days after vaccination and not significantly improving (on clinical judgement), persistent or worsening on repeat testing at a clinically appropriate interval.

If the holding rule has been met and following a safety review by the DSMC it is deemed appropriate to restart dosing or to continue only with the lower dose of vaccine, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The DSMC safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Study Information Booklet (SIB) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The local ethics committee, MHRA, GVGH and the Sponsor will be notified if a holding rule is activated or released.

All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

11.15 INDIVIDUAL HOLDING RULES

In addition to the above stated group holding rule, stopping rules for individual volunteers will apply (i.e. indications to withdraw individuals from further vaccinations):

- **Local reactions:**
  - Injection site ulceration, abscess or necrosis

- **Laboratory AEs:**
  - the volunteer develops a Grade 3 laboratory adverse event considered related within 7 days after vaccination, not significantly improving (on clinical judgement), persistent or worsening on repeat testing.

- **Solicited adverse events:**
  - the volunteer develops a Grade 3 systemic solicited adverse event considered related within 7 days after vaccination (day of vaccination and six subsequent days), persisting continuously at Grade 3 for > 48hrs.

- **Unsolicited adverse events:**
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, persisting continuously at Grade 3 for > 48hrs,
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, which is considered a serious adverse event as assessed by the CI-designated clinician / investigator,
  - the volunteer has a serious adverse event considered related to vaccination, or
  - the volunteer has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.

If a volunteer fulfils any of the temporary exclusion criteria (see section 7) at the scheduled time of a second administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol (see Table 1) or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated volunteers will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

11.16 STOPPING RULES

The trial will be discontinued in the event of any of the following:

- New scientific information is published to indicate that subjects in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the subjects enrolled in this or any other on-going trial of the GMMA vaccine delivery system.
- For any other reason at the discretion of the Chief Investigator or DSMC.

Additionally, the DSMC can temporarily pause the trial if time is required to reach a decision regarding stopping the trial e.g. to determine causality for SAE.

12 STATISTICS

12.1 DESCRIPTIVE STATISTICAL METHODS

The analyses for this study will be descriptive in purpose and will not include any hypothesis testing or presentation of p values for group comparisons or power calculation.

12.2 THE NUMBER OF PARTICIPANTS

30-42 participants will be recruited to the study allocated to groups 1-3 as detailed in section 7. Participants will be replaced only if they have not received a dose of vaccine. There has been no formal power calculation to determine this figure as the study is primarily descriptive. The number of participants has therefore been chosen to pragmatically reflect logistical and budgetary constraints.

12.3 THE LEVEL OF STATISTICAL SIGNIFICANCE

There will be no statistical significance testing. All confidence intervals for descriptive analyses will be set at 95%.

12.4 CRITERIA FOR TERMINATION OF TRIAL

The Chief Investigator and Data Safety Monitoring Committee will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the investigator will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the MHRA, GVGH and relevant Ethics Committee will be notified within 15 days of this occurring.

In the event of the trial being terminated early, follow-up of enrolled participants will still continue as detailed in tables 2.1 for safety reasons, with the exception that further vaccination will not be given and study procedures will be modified to monitor safety only.

12.5 PROCEDURE FOR ACCOUNTING FOR MISSING, UNUSED, AND SPURIOUS

All available data will be used in the analyses and there will be no imputations for missing data. Participants will be analysed according to the group to which they were assigned.

12.6 INCLUSION IN ANALYSIS

All participants with any available data will be included in the analyses.

12.7 INTERIM ANALYSIS

An interim analysis of the secondary objective, immunogenicity at two dose levels (lower dose and full dose iNTS-GMMA Vaccine) will be performed once enrolement is complete and once all second vaccine + Day 28 (Day 84; V8) samples have been collected from all groups. An interim analysis of some of the exploratory objectives may also be performed dependant on validation of the exploratory assays.

13 DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

The investigators will populate the content of participants' CRFs, which will be in a paper and/or electronic format using an REDCap database (or an appropriate alternative). This database is stored on a secure University of Oxford server and has restricted access and is password-protected with accountability records. This data includes safety data, laboratory data (both clinical and immunological) and outcome data. All information transcribed to and from the REDCap database is by encrypted (Https) transfer.

Each study participant will have a unique screening number which will be allocated following the taking of informed consent and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number which will be determined by enrolment order and initials as a second identifier. Samples sent to laboratories for processing will be identified by a trial number and participant number only.

13.1 DATA INTEGRITY

Data collection and storage will be inspected throughout the study by internal (performed by the Oxford Vaccine Group) and external (Appledown Clinical Research Ltd) monitoring. The Sponsor may also audit the trial data.

13.2 DATA ARCHIVING AND STORAGE

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked filing cabinet at the site. All essential documents will be retained for a minimum of 5 years after the study has finished. Volunteers who complete online screening or telephone screening only (before informed consent) will not have data kept beyond the end of the trial. The need to store study data for longer in relation to licensing of the vaccine will be subject to ongoing review. For effective vaccines that may be licensed, we may store research data securely at the site at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for 7 years in line with the site financial policy. De-identified research data maybe be stored indefinitely.

13.3 SOURCE DATA

Source documents are original documents, data, and records from which participants' CRF data is populated. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study CRF entries will be considered source data where it is the site of the original recording. All documents will be stored safely under strict confidentiality and with restricted access. On all study-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the study participant number/code only.

13.4 ACCESS TO DATA

Direct access will be granted to authorised representatives from the sponsor/host institution (including Appledown clinical Research Ltd), GSK/GVGH and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.5 DATA RECORDING AND RECORD KEEPING

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. REDCap, or similar) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment. REDCap is widely-used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored in a separate password protected Access databased saved on a secure University of Oxford server. Only Oxford staff have access to the Access database and are permitted for data entry.

Each study participant will have a unique participant number which will be allocated at the time a screening visit is booked and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number and/or code. Samples sent to laboratories for processing will be identified by trial number and participant number only.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g. requesting to be informed of other trials), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Bank details will be stored for 7 years in line with University financial policy.

14 QUALITY ASSURANCE PROCEDURES

14.1 RISK ASSESSMENT

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

14.2 MONITORING

Regular monitoring will be performed by Appledown Clinical Research Ltd according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

15 PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file as per SOP.

16 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.
A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority, the relevant NHS host organisation and GSK/GVGH within seven calendar days.

17 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 DECLARATION OF HELSINKI

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

17.2 GUIDELINES FOR GOOD CLINICAL PRACTICE

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

17.3 APPROVALS

Following sponsor approval the protocol, informed consent form, participant information sheet and required material will be submitted to an appropriate Research Ethics Committee (REC), MHRA, regulatory authorities, and host institution(s) for written approval.
The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4 TRANSPARENCY IN RESEARCH

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17.5 REPORTING

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and summary report will be submitted to the MHRA, the REC, host organisation and Sponsor.

17.6 PARTICIPANT CONFIDENTIALITY

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number and initials on all trial documents and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

17.7 PARTICIPANT REIMBURSEMENT

Each participant is compensated for their time and for the inconvenience based on the following figures:

- Travel expenses: £15 per visit
- Inconvenience of blood tests: £10 per visit
- Time required for visits: £20 per visit

Remuneration is on a *pro rata* basis should a participant fail to complete all visits and/or study requirements. Each participant can therefore receive a maximum of £585. Payments will be made in instalments after V0, V6, V10, and V12.
Additional reimbursement for unscheduled visits at £45 per visit will be provided. This will not be given unless an unscheduled visit occurs.

18 FINANCE AND INSURANCE

18.1 FUNDING

This clinical trial is funded by a European Union Horizon2020 grant. Additional budget from GVGH will cover necessary costs for monitoring activities not already funded by the Vacc-iNTS European Union Horizon2020 grant.

18.2 INSURANCE

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London

18.3 CONTRACTUAL ARRANGEMENTS

Appropriate contractual arrangements will be put in place with all third parties.

19 PUBLICATION POLICY

The Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator prior to submission.

20 DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP derived from this trial will be in accordance with the Consortium Agreement signed by Beneficiaries of the Horizon2020 Vacc-iNTS EU Grant No 815437.

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APPENDIX A: GRADING THE SEVERITY OF SOLICITED AND UNSOLICITED ADVERSE EVENTS

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6
	1	37.6 – 38.0
	2	38.1 – 39.0
	3	> 39

Adverse event	Grade	Definition
Any symptom	0	Absence or resolution of symptom
	1	Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3	Significant interference with daily activity
	4	Emergency department visit or hospitalisation
	5*	Fatality

*All grade 5 AE will be considered either a SAE, SAR, or SUSAR dependant on causality and 'expectedness'

APPENDIX B: GRADING THE SEVERITY OF VISIT OBSERVED ADVERSE EVENTS

Observation	Grade 1	Grade 2	Grade 3
Oral temperature (ºC)	37.6 – 38.0	38.1 – 39.0	>39
Tachycardia (beats/min)	101-115	116-130	>130
Bradycardia (beats/min)	50-54	45-49	<45
Systolic hyper-tension (mmHg)	141-150	151-155	>155
Diastolic hyper-tension (mmHg)	91-95	96-100	>100
Systolic hypo-tension (mmHg)	85-89	80-84	<80

The following ranges are considered normal physiological ranges and are recorded as Grade 0:

- Oral temperature between 35.5 and 37.5 C
- Resting heart rate between 55 and 100 beats/minute
- Systolic blood pressure between 90 and 140 mmHg

APPENDIX C: GRADING THE SEVERITY OF LABORATORY OBSERVED ADVERSE EVENTS

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
Haemoglobin: decrease from baseline value (g/l)	≤ 15	16-20	21-50	>50
White cell count: elevated (10^9/L)	11.5–15	>15–20	>20–25	>25
White cell count: depressed (10^9/L)	2.5-3.5	1.5-2.49	1.0-1.49	<1.0
Neutrophil count (10^9/L)	1.5-1.99	1.0-1.49	0.5-0.99	<0.50
Platelets (10^9/L)	125-140	100-124	25-99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150
Potassium: hyperkalaemia (mmol/L)	5.1–5.2	5.3–5.4	5.5–5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.3–3.4	3.1–3.2	3.0	<3.0
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	114-156	157-312	>312	RRT
ALT and/or AST (IU/L)	1.1–2.5 x ULN	>2.5–5.0 x ULN	>5.0-10 x ULN	>10 x ULN
Bilirubin, with increase in LFTs (µmol/L)	1.1–1.25 x ULN	>1.25–1.5 x ULN	>1.5–1.75 x ULN	>1.75 x ULN
Bilirubin, with normal LFTs (µmol/L)	1.1–1.5 x ULN	>1.5–2.0 x ULN	>2.0–3.0 x ULN	>3.0 x ULN
Alkaline phosphatase (IU/L)	1.1–2.0 x ULN	>2.0–3.0 x ULN	>3.0–10 x ULN	>10 x ULN
Albumin: hypoalbuminaemia (g/L)	28–31	25–27	<25	Not applicable
C-reactive protein	>10-30	31-100	101-200	>200

Grade 4* Potentially life threatening

APPENDIX D: WHO CLINICAL PROGRESSION SCALE FOR CLINICAL STUDIES OF COVID-19

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

APPENDIX E: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3	14/06/2022	Brama Hanumunthadu/Nelly Owino/Maheshi Ramasamy	• Edit to Typhoid / Paratyphoid vaccine as an exclusion criteria • Clarification of SARS-COV-2 test as a temporary exclusion criteria • Addition / modification of mailout language and inclusion of use of GP databases to identify potential participants
NSA01	3.1	13/01/2023	Timothy Crocker-Buque	• The protocol has been has been edited to clarify: • Minor edit to Participant study windows for the V5, V8 and V11 (D28 post vaccination Visit). Edit changed from D28 +/- 4 days to +/- 7 days. This change has been made to improve participant management and Data collection.

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC / MHRA / HRA submission.
Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, HRA (where required) or MHRA.

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Sal*monella* **V**accine Study in **O**xford

SALVO

INFORMED CONSENT FORM

Participant's Name: _____

Participant Initials: _____

Participant Number: S A L ___ ___ ___

If you agree, please initial box:

Section 1: Study Procedures	
1. I confirm that I have read the information sheet dated..... (version.....) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I have spoken with Dr/Nurse_____	
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
4. I have received detailed information about the intervention schedule, study procedures, potential side effects and their importance.	
5. I agree to be randomised to receive either the iNTS-GMMA vaccine (Lower or Full Dose Schedule) or the placebo schedule as detailed in the participant information sheet and I am aware of the risks and side effects associated with each intervention. I am aware that each schedule includes 3 vaccine or placebo administrations.	

6. I will bring the 24-hour contact reply slip, signed by my 24-hour contact prior to receiving the first dose of vaccine or placebo. I agree that the study team may contact this person if I cannot be contacted during the study.	
7. I agree to refrain from donating blood/blood products for the duration of the study.	
8. ***Women only***: I understand the need to ensure that I or my partner use effective contraception one month prior to first vaccination and continue to do so for the remainder of the study.	
Section 2: Personal Information	
9. I agree to OVG storing and using my personal information as described in the information booklet.	
10. I agree to my General Practitioner being informed of my participation in this study. I agree to my GP and/or other treating doctors being approached for additional information regarding my medical and vaccination history and study staff to access my NHS medical records either via my GP or the electronic patient records system.	
11. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University of Oxford (Sponsor), from regulatory authorities [and from the NHS Trust(s)], where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I agree to my National Insurance (if UK citizen) or Passport number being used to register me on TOPS. I understand that it will be stored electronically for the duration of the study.	
12. I understand TOPS is a Health Research Authority database that aims to prevent healthy volunteers from taking part in too many studies. I understand that only staff at OVG and other research units can use the database and OVG may call other units, or OVG may be called, to check volunteer details.	
13. I agree to provide my bank account details including my account name, sort code and account number for reimbursement purposes. I understand that my banking details will be stored electronically as described in the information booklet. I understand that my personal information will be shared to the extent required to process or verify eligibility of payments as described in the information booklet.	
Section 3: Research Samples and Data	
14. I agree to donate blood and saliva samples. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	

15. I agree to my de-identified data and biological samples being sent and stored within and outside of the European Union for analysis by collaborating research groups as described in the information booklet.		
16. I understand and agree that some of my samples will be used to investigate the genetic factors determining the response to iNTS-GMMA vaccine or placebo.		
If all of the applicable sentences above are initialled, meaning "yes", then please continue:		
17. I agree to take part in this study.		
Optional:		
18. I agree to donate stool samples for this study. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	Yes	No
19. I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacted does not oblige me to participate in any further studies.	Yes	No

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 original for researcher site file.*

OXFORD VACCINE GROUP

Sal*monella* **V**accine Study in **O**xford
SALVO

PARTICIPANT INFORMATION SHEET

You are invited to take part in a study to test a new vaccine against Invasive Non-Typhoidal *Salmonella* (iNTS), an important cause of blood poisoning in children and adults in sub-Saharan Africa. The study is being run by the Oxford Vaccine Group, which is part of the University of Oxford.

Participation in the study is entirely voluntary. Before you decide whether to take part, it is important for you to understand what the study is about and what participation would involve. Please take time to read the information carefully and discuss with others if you wish. If anything is unclear or you would like further information, please contact the study team.

Thank you for taking the time to consider taking part in this study.

Contact Details
Oxford Vaccine Group
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital
Oxford OX3 7LE
Tel: 01865 611400
Email: info@ovg.ox.ac.uk
Website: www.ovg.ox.ac.uk

Who are the Oxford Vaccine Group?

The Oxford Vaccine Group, which is part of the **University of Oxford**, is an independent research team of doctors, nurses and play assistants. We carry out research studies of new and improved vaccines for babies, young children, teenagers and adults, and teach doctors and nurses about immunisations. In the past 5 years alone, over 7,000 participants in the Thames Valley area have taken part in our research studies.

What is invasive Non-Typhoidal *Salmonella* disease?

Non-typhoidal *Salmonella*e are a group of bacteria, that are well known to cause food poisoning throughout the world. However, in certain circumstances they can cause a more serious disease, where *Salmonella* can spread beyond the gut leading to blood poisoning, and in some cases sepsis and death. This is called invasive non-typhoidal *Salmonella* (iNTS) disease and is of particular concern in individuals with a weaker immune system. iNTS disease occurs in over half a million people a year, and particularly affects children under 5 years of age in sub-Saharan Africa, where it causes significant disease with over 200,000 cases and 31,000 deaths per year. Adults can also be affected by iNTS disease, particularly those with HIV, malaria or malnutrition.

What is the purpose of the study?

In this study we are investigating a new vaccine against iNTS called the iNTS-GMMA vaccine. This new vaccine is developed by the GSK Vaccine Institute for Global Health (GVGH), a GlaxoSmithKline (GSK) company based in Italy with which the Oxford Vaccine Group is collaborating for the development of a vaccine against iNTS. This will be the first time this vaccine will be given to human volunteers. It contains small amounts / particles of the outer surface of the two most common bacteria that cause iNTS disease (*Salmonella* Enteritidis and *Salmonella* Typhimurium). These particles previously known as Generalised Modules for Membrane and Antigens', and currently abbreviated to 'GMMAs', constitute the main component of the vaccine. **The vaccine does not contain *Salmonella* bacteria and therefore cannot cause infection or disease**. It is hoped that these GMMA particles can stimulate the immune system to produce a protective response against iNTS bacteria and thus prevent future blood stream infections by these bacteria. The GMMA particles are diluted in Alhydrogel, a common vaccine component designed to reduce local side effects. The study is being conducted to evaluate the safety of the vaccine and how well it stimulates the immune system against iNTS.

Not everyone will receive the active vaccine, some individuals will receive a placebo (non-active comparison). The placebo contains all the components of the iNTS-GMMA vaccine except for the active GMMA particles i.e. Alhydrogel alone and cannot stimulate the immune system to produce a protective response against iNTS bacteria. Participants will be enrolled sequentially into 3 groups and randomly allocated to receive either the iNTS-GMMA vaccine or the placebo. Randomization means that neither you nor your doctor will choose whether you receive the active vaccine or placebo. In this study, a computer will assign this to individual participants, like flipping a coin. You have an equal chance of receiving the vaccine versus placebo in groups 1 and 2 and have more chances of receiving the vaccine than the placebo in group 3.

Why have I been invited?

We are inviting healthy adults aged 18 to 55 years old to take part in this study. We would not want to recruit anyone who has significant health issues, anyone with altered immune function or any females who are pregnant or thinking of starting a family soon. We use various ways to contact potential volunteers, including the Electoral Roll and the National Health Applications and Infrastructure Services (NHAIS) who hold the central NHS patient database (Open Exeter). This database identifies all persons within the local area who are in the appropriate age range. Whilst we do commission the invitation to take part in the study, we do not have access to personal data and are not directly responsible for the mail out. In addition, you may have previously expressed an interest in taking part in studies at the Oxford Vaccine Group.

What should I consider?

We are keen to recruit healthy volunteers who are:

- Willing to take part and able to attend all study visits
- Aged between 18 and 55 years old, inclusive
- Willing to allow us to communicate with their GP to notify them of your participation in the study and to check your medical history
- (Females) Willing to use effective contraception from 1 month prior to vaccination and for the remainder of the study

Do I have to take part?

No. We are looking for volunteers. Should you volunteer and later change your mind (for whatever reason) it is your right to do so, and you would not need to provide an explanation to the study team or anyone else. In addition, your decision to withdraw would not affect any ongoing medical care you are or will be receiving.

Whatever you choose it's important that you are happy with your decision and it is not the role of the study team to decide for you. If you choose to withdraw after the receiving the vaccine, we would stop all research related activities. However, we would like to check that you remain well after receiving the vaccine for your own safety which may or may not include follow up visits / blood tests. We would use the samples and data we have collected from you in our analysis of the study, up until the point you informed us that you wanted to withdraw.

What are the possible benefits of taking part?

There are no clear benefits to you if you take part in this study. However, you would have the knowledge that you played a part in the early stages of developing a new vaccine against a bacteria that causes a significant burden of death and disease, particularly in sub-Saharan Africa and in children under 5 years of age for which there are currently no licensed vaccine.

Are there any possible disadvantages or risks from taking part?

In general, the risks are in relation to the vaccine/placebo, blood and oral fluid sampling. In addition, you would be asked to attend regular visits at the CCVTM. In regards to COVID-19 please see the COVID-19 section below.

- **Vaccine**

General Vaccines

Intra-muscular vaccination can commonly cause reactions, although most tend to be minor and only last a few days. These may typically include injection site - discomfort, redness, and swelling. As for all vaccines some volunteers occasionally may feel generally unwell, develop fevers, muscle aches, joint aches, headache, experience loss of appetite, nausea / vomiting, abdominal pain or diarrhoea. Not everyone will experience symptoms and if they do occur, they should resolve after a few days.

Anaphylaxis is a very rare but a potentially life-threatening allergic reaction and may occur after immunisation. All clinical staff are trained in the immediate treatment of anaphylactic reactions including the use of intra-muscular adrenaline. It is for this reason you need to wait at least 60 minutes after each vaccine dose is given, as this would be within the typical time frame should this reaction occur.

iNTS GMMA Vaccine

This study is the first time that the iNTS-GMMA vaccine will be given to human participants. The studies performed in animals prior to moving on to human trials have shown good safety results. In addition, GMMA-based vaccines against other bacteria have been safely used in over 190 volunteers. These vaccines were found to be well-tolerated and safe in the volunteers who received them. Nevertheless, this is a new vaccine and there may be side effects we do not know about. It is important for you to be aware of this. However, we have multiple measures in place to ensure your safety during the trial as outlined below.

Throughout the study, the safety of the participants in all groups will be monitored following vaccination. This will be done by reviewing of symptoms at visits and through the electronic Diary (eDiary). For your safety there will be an independent safety committee consisting of an independent panel of experts known as the Data and Safety Monitoring Committee (DSMC) who will know whether you have received vaccine or placebo in order to periodically monitor the overall safety of the trial. This committee will review the safety data particularly as the trial proceeds between Groups 1, 2 and 3 and would be required to approve progression from lower to full dose active iNTS-GMMA vaccine within the trial. Should there be any concerns this committee has the power to halt the trial for further evaluation. In addition, you will be provided with study team contact details who are available 24/7 should you need to contact us.

We would ask you to provide contact details of a person who would act as a second contact. Only to be used in an emergency or needing to contact you urgently.

- **Placebo**

The placebo (Alhydrogel) is a common component used in many vaccines and would not usually cause any side effects other than the ones known for any general vaccine as listed

above. Not everyone will experience side effects and if they do occur, they should resolve after a few days.

- **Blood/Oral Fluid Sampling**

Blood tests can be painful and sometimes leave bruising and/or temporary discomfort, but these all resolve in a very short period of time. Rarely fainting can occur. Oral fluid samples are collected with a cotton swab of the mouth and should not cause any discomfort.

- **Pregnancy**

For females, you should not take part in this trial if you are pregnant or breastfeeding. It is currently unknown whether the vaccine being tested is safe during pregnancy. For this reason, it is important that women use adequate contraception during the study period. Women who are not of childbearing potential (i.e. postmenopausal or permanently sterile due to surgery such as a hysterectomy) will not be required to use contraception. This will be discussed with you at the screening visit. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the trial, although we will ask to follow you up for safety reasons.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible.

- **COVID-19**

It is difficult to predict the time course of the COVID-19 pandemic. Should further peaks occur during the study, we will implement specific measures to ensure your safety whilst taking part in the study. **The safety of our study participants remains the prime consideration during the trial.** If you have not already received a COVID-19 vaccine and you become eligible (according to UK policy) for the vaccine during the study, we would talk to you about this. If you agreed and if it was possible, we would find a mutually agreeable time for you to receive this vaccination. We would not prevent you from receiving this vaccination. During the study we will follow up-to-date government advice including on advising testing, self-isolation, and personal protective equipment (PPE) as necessary. Should you develop symptoms of COVID-19 or test positive for COVID-19 during the study, we would want to know about this prior to coming to clinic. This may affect the timings of your clinic visits and we would want to assess whether it is safe for you to receive further study vaccinations.

What will happen to me if I decide to take part?

- **Recruitment**

If you express an interest in taking part, a member of the Oxford Vaccine Group will contact you by telephone to discuss the study and answer any questions you may have. If you have accessed the participant information sheet via the online questionnaire you will have already answered initial eligibility questions. We would like to ask you a few more detailed questions to assess your eligibility.

Following this, if you are interested and seem suitable for the study then we would arrange for you to come to our clinic for a screening visit. In addition we would send you a consent

form (paper or electronic) giving permission for the study team to access your medical records to obtain this information via the electronic patient records or through your GP. We would then ask you to return a copy of the signed consent form (paper or electronic). A countersigned form will be provided at the screening visit. This consent form is only to allow access to your medical records, and not the consent for enrolment on to the study. If you choose to participate in the study a separate consent will be taken for inclusion into the trial.

- **Screening Visit**

The **purpose of screening tests is to ensure that you are eligible to take part** and that by taking part in the study you are not taking on any extra risk to your health. At the screening visit we would sit with you and go through the study in detail. This visit would provide an opportunity for you to ask any questions you might have about the study and what's involved. You would be allowed as much time as you feel necessary before making any decision on whether to take part.

Is coming to screening a commitment to taking part?

No. It's an opportunity to meet with the study staff and ask questions; you do not need to make a decision there and then.

What if I wish to volunteer?

If you are keen to proceed, we would ask you to sign an **informed consent form**. Only once this is signed would we then start any study procedures.

What are the study procedures at the screening visit, if I decide to volunteer?

We would ask you questions about your health, undertake a physical examination and take a blood sample (of 10ml; approximately two tablespoons). We would also require a urine sample. Blood testing includes HIV and hepatitis B and C tests, as well as screening for anaemia, liver and kidney function. For females, we would perform a pregnancy test on your urine sample.

Demographic data including your name, date of birth, gender, ethnicity and contact details will be collected, if not already recorded at the recruitment stage. Data protection regulation governs how we store and process your data. Please see 'What will happen to my data?' below for further information.

We would also seek your consent to register your name on the 'The Over-volunteering Prevention System' (TOPS) national database. This is designed to guard against the potential for harm that can result from excessive volunteering in clinical trials involving investigational medicinal products and blood donations. This would be done using your National Insurance number or passport number, and all information is kept confidential. More information can be found at http://www.hra.nhs.uk/about-the-hra/our-committees/the-over-volunteering-prevention-system/. If we have not already done so, prior to your screening visit we would also ask for your consent to access your medical records via the electronic patient record system or via your GP to obtain any relevant medical history that may affect your participation in the study. Once the study team have confirmed your suitability for the trial, we would inform you and arrange a date for your

first visit. You would be formally enrolled into the study at the time the first vaccine dose is given.

- **Enrolment**

If you are eligible for the study, you will be enrolled into 1 of 3 groups (outlined below) dependant on your enrolment date:

Group 1	Randomly allocated 1:1 to receive either the lower dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 2	Randomly allocated 1:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 3	Randomly allocated 2:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to eighteen participants will be allocated to this group.

Table 1: Enrolment of Groups 1-3

- **Overview of Study Visits**

Each participant would receive 3 vaccinations with the iNTS-GMMA vaccine or placebo at intervals of 0, 2 and 6 months. In addition, each participant would require blood tests immediately before each vaccination and at specific intervals after each vaccination. The study will require a total of 12 visits over a 12-month period.

A simplified overview of the study is shown in the diagram below:

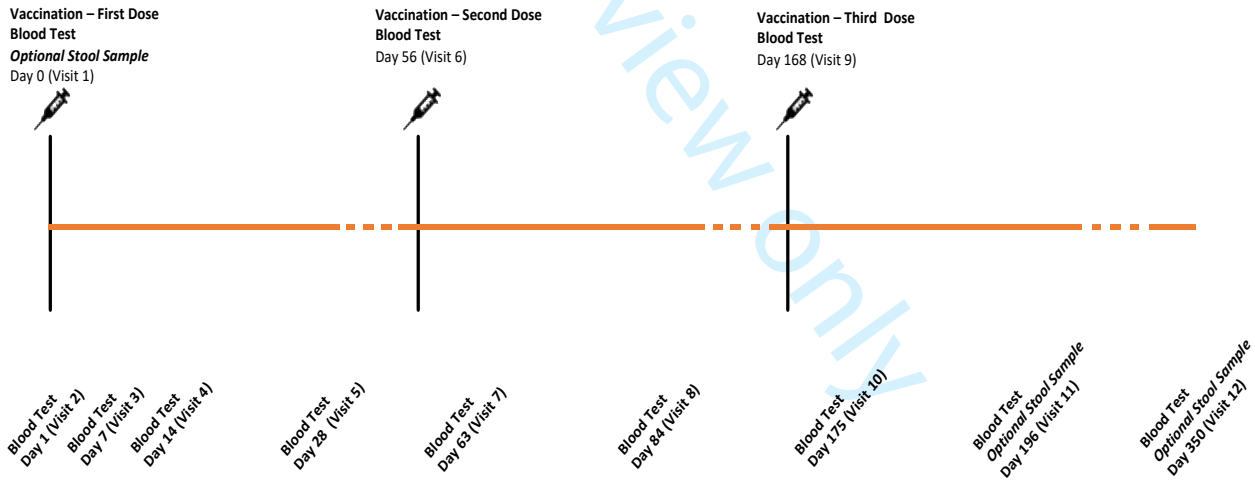

Figure 1: Overview of Study Design

Neither you nor the study team analysing the data will know whether you have received the iNTS-GMMA vaccine or the placebo. This is termed a 'participant-observer blind' trial and provides greater confidence in the final conclusion of the study.

*For participants in group 3 we will collect an additional blood sample (30mls, equivalent to 6 tablespoons) to develop a serum standard at Visit 8 (Day 84). The purpose of the serum

standard is to create a supply of antibodies directed towards the iNTS-GMMA Vaccine that can be used as a reference standard to develop laboratory tests and compare how well this vaccine or future vaccines are working in the individuals being vaccinated. Due to the study design participants who receive the placebo will also donate this additional sample. While these samples will undergo the same laboratory tests, these samples will not be used as a reference standard but may be used in laboratory test development.

- **Vaccine Visits (Visit 1, 6 and 9)**

You would be given a date, time and place to come to the Oxford Vaccine Group at the Churchill Hospital. We would start by checking that you are happy to remain in the study and ask if anything has changed medically since we last saw you.

We would check your pulse and blood pressure at every visit, and for all women a pregnancy test would be done prior to administering each dose of vaccine or placebo. We would take blood and oral samples and give the first vaccine by injection into the muscle of the upper arm.

After vaccination you would need to wait with us for 60 minutes before leaving. This is standard practice to monitor any reactions to the vaccine.

We would give you a tape measure, thermometer and set you up on the **electronic diary** for you to record any symptoms or side effects and daily temperatures for the next **7 days following vaccination**. We would explain how you record this information when you are with us, and it should be entered electronically via a secure link and encrypted transfer on our website wherever possible. Monitoring the ediary will allow the team of research doctors and nurses to check on your wellbeing and ensure your safety.

- **Follow-up Visits**

There are two to four scheduled follow up visits following each vaccination. These visits usually last approximately 30 minutes. We would check on your progress during the trial, review any ediary entries, take recordings of your pulse and blood pressure and collect any blood or oral fluid samples as per the sample schedule below.

- **Samples**

We collect a blood sample at each visit. The amount will be between 5mL (about 1 tablespoon) and 70.5 mL (quarter of a cup) depending on the tests that will be done. The total amount of blood taken during the whole study (1 year) would be up to 667.5mL (about 3 cups), which is significantly less than the maximum amount of blood that could be donated over a year to the UK Blood Donation Service. This is in line with the blood donation guidelines and your body would replace this naturally after about three months. Repeated blood tests can cause anaemia (low haemoglobin), however we have checks and processes in place to ensure this is minimised, including reducing the blood volume we take from you. In rare cases we may ask you to see your general practitioner for a medical review. These blood tests are important to measure how good the vaccine is at producing an immunity to iNTS. In addition, we periodically check your general blood tests including full blood count, urea and electrolytes and liver function tests to ensure they are normal throughout the study.

At some visits we will collect an oral fluid sample using a simple 10 swab (1-2 minutes). In addition, to blood we are testing oral fluid to measure indicators of immunity to iNTS.

In addition, stool samples may be collected during the study. This is entirely **optional**. If you do not agree to the collection of stool samples, this will not affect your participation in the study. If you opt-in, the stool samples will be collected before the first vaccine is administered (Visit 1), 28 days after the third vaccine (Visit 11) and at the end of the study (Visit 12). We will test for the microscopic organisms such as bacteria, parasites and fungi that naturally occupy your gut to see if the mix of these microscopic organisms influences your response to the study vaccine. In addition we will test for the development of gut antibodies to Non-typhoidal *Salmonella*e. You will be given collection materials and the study team will explain how to collect the samples. We will receive the samples at the specified visits. However, if you are unable to provide a sample there is an option to free post if you are happy to do so. You will be given 'By Post' collection materials and the study team will explain how to collect and post the sample.

There might be times when your blood needs to be retested or a urine sample taken to confirm test results. This might happen at your next visit or at an unscheduled visit that you would need to come to the clinic for. At this visit the study staff would take a sample of your blood (usually 10 mL, 2 tablespoons) and collect a urine sample, as needed.

What will happen to the samples I give?

Your sample will be assigned a code and your data will also be identified only by this number. The material given to researchers will not have information that directly identifies you. The blood, urine, stool and oral fluid samples collected during this study would be analysed in the Oxford University Hospitals, University of Oxford research laboratories, while some blood samples will be shipped to the GSK Vaccine Institute for Global Health (GVGH), Siena, Italy or delegated laboratories based in Italy. We would also send some samples to other researchers working with us on this research project, including researchers outside the European Union. Your samples will be identified by a code instead of your name and the link between your name and the code number will not be shared with external companies and laboratories.

If you choose to take part in this study, we will be asking for your separate permission to store blood (including cells and DNA) samples, in a collection of samples called Biobank. Details of this will be provided in a separate booklet after you are enrolled into this study, and you are free to say no to the Biobank and continue to take part in this study if you wish. If you do not wish for your samples to be stored in the Biobank, they will be destroyed 12 months after the last participant has completed the study.

What if any of my test results were abnormal?

If there are any abnormal results or undiagnosed conditions found in the course of the study these would be discussed with you and, if you agreed, your GP would be informed of these results. We would not report them to anyone else without your permission. For example, a new diagnosis of high blood pressure might be made. Any newly diagnosed conditions would be looked after by your GP.

Reimbursement

There are no costs for you to participate in this study. Study participants would be reimbursed for their time, travel and inconvenience of taking part in the study. The maximum reimbursement for any volunteer who completes the whole study is £585. All participants will be reimbursed based on the following figures:

Travel expenses: £15 per visit
Inconvenience of blood tests: £10 per blood donation
Time required for visits: £20 per visit

The sum reimbursed is on a pro rata basis, so, if for example, you choose to withdraw halfway through the study we would calculate your reimbursement based on the visits you have attended and samples that have been obtained.

Payments are made directly by bank transfer in instalments during the study. For this reason, we would require participants to provide their bank details at screening. Bank details would be kept confidential. Personal information such as your name, bank details and national insurance number may be shared with the University finance team to process or verify your reimbursement payments. Financial auditors may also audit the records where this information is held. All confidential data will be stored according to the UK General Data Protection Regulation (see below).

You may also receive reimbursement for any unscheduled visits you attend (if you have symptoms from the vaccine and need to be assessed). You would be reimbursed £45 per unscheduled visit, up to a maximum of £135 (equivalent of 3 unscheduled visits). If you do not require any unscheduled visits, you will not be reimbursed for this amount.

Would my taking part in this study be kept confidential?

Yes. All information that is collected about you during the course of the research would be coded with a study number and kept strictly confidential. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. You can search this website at any time. The website will include a summary of the research study results, but it may be many years before research results are posted. Any information about you that leaves the clinic would have any identifiable information removed so that you could not be recognized, with the exception of letters sent to your own GP. In order to enrol into this study, you would be required to sign a form, documenting that you consent for us to contact your GP. This is to inform him/her that you would be entering the study, and to ensure there are no medical reasons that would prevent you from taking part in this study. No one else would be told that you are involved in the study. We would only notify your GP of the results from any medical tests we performed with your permission.

Your information would be stored on a secure server, and paper notes would be held by the Oxford Vaccine Group in a locked cabinet. Your data is retained in case we need to contact you regarding any study related matters or if you wish to contact us regarding your participation in the study. We may also contact you to inform you of future related studies.

Responsible members of the University of Oxford and the Oxford University Hospitals NHS Foundation Trust may be given access to data for monitoring and/or audit of the study to ensure that the research is complying with applicable regulations. In addition, the following groups may inspect the study records without violating your confidentiality:

- Monitors who check that the study is being conducted to a high standard, including the Data and Safety Monitoring Committee (DSMC), an independent panel of experts responsible for trial safety and the Medicines and Healthcare Products Regulatory Agency (MHRA).

Coded data and samples would be sent to other researchers working with us on this research project, including researchers outside the European Union. Please note that your blood samples contain cells and DNA. Your DNA is unique to you so it can never be completely anonymous.

What will happen to my data?

Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' The University of Oxford is the data controller and is responsible for looking after your information and using it properly.

We will be using information from you and your medical records in order to undertake this trial and will use the minimum personally identifiable information possible. We will keep identifiable information about you such as contact details for a minimum of 5 years after the trial has finished. The need to store this information for longer in relation to licensing of the vaccine will be subject to ongoing review.

Paper notes will be held by the Oxford Vaccine Group in a locked cabinet. Once the trial has been completed, all documents, including personally identifiable data, would be archived in a secure facility, for a minimum of 5 years. Storage of this data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. If you complete online or telephone screening, and do not progress to in-person screening, your data will only be stored until the end of the trial.

If you have agreed that samples can be retained for future research then your personally identifiable information will be kept with restricted access solely for the purposes of sample management for a minimum of five years after the last sample has been either used or disposed of in order to meet regulatory requirements. Samples will be provided for future research only in a form that does not identifies you. We store research data securely at the University of Oxford indefinitely following removal of identifiable information.

The trial team will use your name and contact details, to contact you about the clinical trial, and make sure that relevant information about the trial is recorded for your care, in relation to your health during the trial and to oversee the quality of the trial. At the completion of the trial, unless you consent otherwise (e.g. if you request to be informed of other trials),

your personal details will not be used to contact you other than exceptional circumstances concerning your safety.

If you consent to take part in another trial carried out by the Oxford Vaccine Centre, we will retain a copy of your consent form until such time as your details are removed from our database but will keep the consent form and your details separate. Personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

Your bank details will be stored for 7 years in line with university financial policy.
Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Professor Andrew J Pollard, or his successor, as Director of the Oxford Vaccine Group will have the responsibility for custody of the data.

Further information about your rights with respect to your personal data is available at:
https://compliance.web.ox.ac.uk/individual-rights

If you withdraw from the trial, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.

You can find out more about how we use your information by contacting Oxford Vaccine Group on 01865 611400 or email info@ovg.ox.ac.uk.

What will happen at the end of the research study?

The results of the research will be published in a scientific medical journal and potentially presented at future conferences; this can potentially take a few years. All OVG publications will appear on the OVG website and you will receive a letter containing these results. Your individual results would not be identifiable, nor would you be identified in any report or publication. The results of the research will also potentially be used for future academic research within the Oxford Vaccine Group. Some of the research being undertaken will also contribute to the fulfilment of an educational requirement (e.g. a doctoral thesis).
Once the last laboratory test is performed in the study, all samples will be destroyed, unless you have consented for them to be transferred to the Biobank. If your samples are going to the Biobank, a copy of your informed consent form (which contains your personal information), are stored with those samples.

What if there is a problem?

If you have private medical insurance, you are advised to contact your insurance company before participating in this trial. The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this study.

Where can I get advice on whether to take part?

We are happy to answer any questions you might have and contacting us does not commit you to taking part in the study.

Other useful links for general information on taking part in research include:

- www.crn.nihr.ac.uk/can-help/patients-carers-public/how-to-take-part-in-a-study/
- www.nhs.uk/Conditions/Clinical-trials/Pages/Introduction.aspx

What if I wish to complain?

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Professor Andrew Pollard, Director of the Oxford Vaccine Group, (Tel: 01865 611400, Email: info@ovg.ox.ac.uk) or you may contact the University of Oxford Research Governance, Ethics and Assurance (RGEA) office on 01865 (6)16480 or the Head of RGEA, email ctrg@admin.ox.ac.uk.

At any time during the study you would be entirely free to change your mind about taking part, and to withdraw from the study. This would not affect your subsequent medical care in any way.

Who is funding the study?

The study is funded by an EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant, as part of a wider project to progress the iNTS-GMMA vaccine initially through the SALVO clinical trial with a further study to take place in sub-Saharan Africa. For further information on the Vacc-iNTS project please see: https://vacc-ints.eu

Independent monitoring of the study will be undertaken by Appledown Clinical Research Ltd which will be funded by GSK Vaccine Institute for Global Health (GVGH).

Who has reviewed and approved this study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given a favorable opinion by South Central - Oxford A Research Ethics Committee. In addition, this study has been reviewed by the Medicines and Healthcare Regulatory Agency (MHRA) the UK agency responsible for ensuring that medical products under investigation (in this case the active vaccine) is safe and appropriate to continue to clinical trial.

In summary, what would happen if I decide to take part in the study?

- We would ring you to check it is appropriate to include you in the study.
- You would then attend a screening visit in our department (CCVTM).
- At the screening visit we would go through the study in detail and answer any questions you may have. If you are happy to proceed, we would then ask you to sign a consent form. You would then have a brief medical assessment including a medical

history and physical examination. You would have a blood test and urine test (and a pregnancy test for women). These are to assess your eligibility for the study.

- Following satisfactory screening results confirming your eligibility we would invite you to the initial vaccination visit (V1) and enroll you into the study.
- You would be vaccinated with the study vaccine or placebo, once enrolled, then at month two and six.
- You would be seen two to four times after each vaccination; with a final visit one year after the first vaccination. These will include a blood test +/- an oral fluid sample.
- You would be required to fill in an eDiary for seven days after each vaccine.
- The study duration is approximately one year at which time you will be seen 12 times at the CCVTM (not including unscheduled visits or the initial screening visit).

What do I do now?

Thank you for considering taking part in this study. You do not need to make a final decision straight away. If you wish to discuss any element of the study further, then please contact us by either

- telephone **01865 611400**
- website: http://trials.ovg.ox.ac.uk/trials/salvo
- email: info@ovg.ox.ac.uk

Dr Maheshi Ramasamy
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Senior Research Nurse

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supp Material – Trial Summary
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Supp Material

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Supp Material
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Supp Material
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Supp Material
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	5

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Supp Material
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Supp Material
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5 Supp Material
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Supp Material

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6 Supp Material In Blinding and Randomisation Plan
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Supp Material In Blinding and Randomisation Plan
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Supp Material In Blinding and Randomisation Plan
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Supp Material In Blinding and Randomisation Plan
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Supp Material In Blinding and Randomisation Plan
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	Supp Material

		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Supp Material
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Supp Material
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8 Supp Material
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8 Supp Material
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8 Supp Material

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Supp Material
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Supp Material
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Supp Material
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Supp Material
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Supp Material
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Supp Material
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Supp Material
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	Supp Material

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Supp Material
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Supp Material
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA – no intended use of professional writers
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Protocol – as part of submission
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supp Material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp Material

BMJ Open

Salmonella Vaccine Study in Oxford (SALVO) Trial: Protocol for an Observer-Participant Blind Randomised Placebo-Controlled Trial of the iNTS-GMMA Vaccine within a European Cohort

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-072938.R2
Article Type:	Protocol
Date Submitted by the Author:	29-Sep-2023
Complete List of Authors:	Hanumunthadu, Brama; Oxford University, Oxford Vaccine Group Kanji, Nasir; Oxford University, Oxford Vaccine Group Owino, Nelly; Oxford University, Oxford Vaccine Group Ferreira Da Silva, Carla; Oxford University, Oxford Vaccine Group Robinson, Hannah; University of Oxford Oxford Vaccine Group, Department of Paediatrics; NIHR Oxford Biomedical Research Centre White, Rachel; Oxford University, Oxford Vaccine Group Ferruzzi, Pietro; GSK Vaccines Institute for Global Health Nakakana, Usman; GSK Vaccines Institute for Global Health Canals, Rocio; GSK Vaccines Institute for Global Health Pollard, Andrew; University of Oxford, Paediatrics Ramasamy, Maheshi; Oxford University, Oxford Vaccine Group Vacc-iNTS Consortium, Collaborators; Vacc-iNTS Consortium
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Global health
Keywords:	Clinical Trial, IMMUNOLOGY, Public health < INFECTIOUS DISEASES

Salmonella Vaccine Study in Oxford (SALVO) Trial: Protocol for an Observer-Participant Blind Randomised Placebo-Controlled Trial of the iNTS-GMMA Vaccine within a European Cohort

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Keywords:
Clinical Trial
Non-typhoidal Salmonella
Vaccine
Invasive salmonellosis

Abstract

Introduction

Invasive non-typhoidal Salmonellosis (iNTS) is mainly caused by *Salmonella enterica* serovars Typhimurium and Enteritidis and is estimated to result in 77,000 deaths per year, disproportionately affecting children under 5 years of age in sub-Saharan Africa. Invasive non-typhoidal *Salmonellae* serovars are increasingly acquiring resistance to first line antibiotics, thus an effective vaccine would be a valuable tool in reducing morbidity and mortality from infection. While NTS livestock vaccines are in wide use, no licensed vaccines exist for use in humans. Here, a first-in-human study of a novel vaccine (iNTS-GMMA) containing *S.* Typhimurium and *S.* Enteritidis Generalized Modules for Membrane Antigens (GMMA) outer membrane vesicles is presented.

Method and Analysis

The *Salmonella* Vaccine Study in Oxford (SALVO) is a randomised placebo-controlled participant-observer blind phase 1 study of the iNTS-GMMA vaccine. Healthy adult volunteers will be randomised to receive 3 intramuscular injections of the iNTS-GMMA vaccine, containing equal quantities of *S.* Typhimurium and *S.* Enteritidis GMMA particles adsorbed on Alhydrogel, or an Alhydrogel placebo at 0-, 2- and 6-months. Participants will be sequentially enrolled into 3 groups: Group 1, 1:1 randomisation to low dose iNTS-GMMA vaccine or placebo; Group 2, 1:1 randomisation to full dose iNTS-GMMA vaccine or placebo;

Group 3, 2:1 randomisation to full dose or lower dose (dependant on DSMC reviews of Group 1 and 2) iNTS-GMMA vaccine or placebo

The primary objective is safety and tolerability of the vaccine. The secondary objective is immunogenicity as measured by O-antigen based enzyme linked immunosorbent assay. Further exploratory objectives will characterise the expanded human immune profile.

Ethics and Dissemination

Ethical approval for this study has been obtained from the South Central - Oxford A Research Ethics Committee (Ethics REF:22/SC/0059). Appropriate documentation and regulatory approvals have been acquired. Results will be disseminated via peer reviewed articles and conferences.

Registration Details

EudraCT Number: 2020-000510-14

Article Summary

Strength and Limitations

- SALVO is a first in human study of a novel vaccine against invasive non-typhoidal Salmonellosis (iNTS), a neglected disease largely affecting low- and middle-income countries.
- This study provides the opportunity to investigate the adaptive immune response to bacterial outer membrane antigens, supporting future vaccine development efforts against this disease.
- The volunteers recruited to this trial may not be immunologically representative of the ultimate target population in endemic areas. In addition the vaccination schedule in this study is based on the expected schedule in infants.
- In the absence of a known correlate of protection against iNTS disease, it will not be possible to determine vaccine efficacy in this trial.

Introduction

Non-typhoidal *Salmonellae* (NTS), such as *Salmonella enterica* serovars Enteritidis and Typhimurium, most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens (1). However, some NTS bacterial strains can also cause an invasive syndrome with bacteraemia, high fevers, and metastatic infection which if untreated can lead to septicaemia and death. Invasive non- typhoidal *Salmonella* (iNTS) infections are more common in children, the elderly and in the immunosuppressed, including HIV-infected individuals (2, 3).

The Global Burden of Disease study estimates 535,000 annual cases of iNTS globally, associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella* (1, 4). The highest burden of iNTS disease globally occurs in sub-Saharan Africa, with a pooled annual incidence of 52/100,000 (1, 4). This is likely an underestimate given the limited availability of diagnostics in the region coupled with non-specific disease presentation. The age at which infection occurs shows a bimodal distribution in most African studies with 68.3% occurring in children under 5 years and a second peak in the 30-40 years age group, believed to be associated with HIV, malaria, and malnutrition(3, 5, 6, 7).

Of the invasive pathogens responsible for iNTS in sub-Saharan Africa, *S*. Typhimurium is implicated in approximately two-thirds of all cases, with the ST313 serotype accounting for most isolates (8). In contrast to other non-invasive strains, African ST313 isolates often exhibit genomic degradation and pseudogene formation like that seen in typhoidal *Salmonellae*, which contribute to human host restriction and an invasive phenotype (9, 10, 11, 12). Furthermore, iNTS strains such as ST313 have been associated with multi-drug resistance, leading to *Salmonellae* being classified as WHO high priority antibiotic resistant pathogens (13, 14, 15).

High mortality, logistical difficulties in diagnosing infection in the developing world and increasing antimicrobial resistance strongly advocate for the development of an effective vaccine.

There are no currently licensed vaccines for iNTS although multiple candidates are in early phase development, including O-antigen (OAg) conjugates, oral attenuated vaccines, and multiple antigen display protein-polysaccharide conjugate vaccines. A trivalent iNTS-typhoid vaccine is currently in phase 1 (16).

The investigational product in this study is the iNTS-GMMA vaccine. This novel vaccine developed by GSK Vaccines for Global Health (GVGH) consists of outer membrane vesicles or Generalized Modules for Membrane Antigens (GMMA) of the two most common serotypes associated with invasive disease, *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) (17). Generalized Modules for Membrane Antigens (GMMA) particles contain several immunodominant antigens including the OAg component of bacterial lipopolysaccharide and outer membrane proteins. iNTS-GMMA are immunogenic in animal models, eliciting antibodies directed against OAg and demonstrating serum bactericidal

activity. Immunised animals also appear to have lower systemic bacterial loads on subsequent challenge(18).

This is the first trial to investigate the iNTS-GMMA vaccine in humans. Demonstration of safety and immunogenicity in this study will lead to progression to subsequent studies in a sub-Saharan country of high endemicity.

Study aims and objectives

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine and study the immune response to vaccination. Primary, secondary, and exploratory objectives are detailed in Table 1.

As this is a first-in-human vaccine trial, the primary objective is the safety and tolerability of the iNTS-GMMA vaccine which will be ascertained by the collection of solicited and unsolicited adverse events, serious adverse events, withdrawals and laboratory parameters. Solicited adverse events will be collected up to seven days following each vaccination and include local injection site reactions and systemic symptoms. Unsolicited adverse events will be collected up to 28 days following each vaccination, and outside of this period will be recorded only if medically attended. Serious adverse events and withdrawals will be recorded throughout the study. Laboratory adverse events will be recorded by obtaining blood samples to analyse full blood count, renal and liver profile at Days 0, 7, 28, 56, 63, 84, 168, 175 and 196.

The secondary objective is the determination of immunogenicity of the iNTS- GMMA vaccine. Immunogenicity will be measured using SEn and STm O-antigen specific enzyme-linked immunosorbent assays (ELISA) at timepoints Days 0, 7, 28, 56, 63, 84, 168, 175, 196 and 350.

Objective		Outcome measure
Primary	To determine the safety and tolerability of the iNTS-GMMA vaccine	Clinical review and participant recording of solicited, unsolicited adverse events, serious adverse events, withdrawals, and laboratory parameters (haematology/biochemistry)
Secondary	To investigate immunogenicity of the iNTS-GMMA vaccine	Measurement of serovar specific SEn and STm O-antigen by ELISA before and after vaccination
Exploratory	To further characterise the immune response to vaccination	Exploratory immunological analyses including functional antibody assays and antigen-specific memory B cell responses and T cell responses before and after vaccination

Table 1 Primary, secondary, and exploratory objectives and outcomes of the SALVO study.

Methods

Trial interventions: IMP and placebo

The iNTS- GMMA vaccine consists of outer membrane vesicles or GMMA from the two most common serovars causing invasive disease, *Salmonella* Enteritidis and S*almonella* Typhimurium adsorbed onto Alhydrogel (0.35 mg AL^{3+} /0.5 mL dose) and suspended in isotonic phosphate buffered saline. The parent bacteria have been genetically modified to increase production of outer membrane vesicles (Δ*tolR*) and reduce the toxicity of lipid A component within the lipopolysaccharide (Δ*msbB* and Δ*pagP*). The GMMA particles are filtered and purified to form the active component of the vaccine (19, 20, 21). Two dose levels will be used for this study: a full dose of 20 µg STmGMMA + 20 µg SEnGMMA (total 40 µg O- antigen); and a lower dose consisting of 5.3 µg STmGMMA + 5.3 µg SEnGMMA (total 10.6 µg OAg). The placebo matches the vaccine matrix and consists of Alhydrogel without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

Study design and setting

This is a first-in-human randomised placebo-controlled participant-observer blind trial of the iNTS-GMMA vaccine in healthy adults aged 18-55 years in the United Kingdom. A total of 30-42 participants will be randomised to receive 3 intramuscular doses of active vaccine or placebo at 0, 2 and 6 months (Figure 1). For further details please see SALVO Protocol in Supplementary Material 1.

Figure 1 SALVO vaccine and visit schedule

Participants will be sequentially enrolled into three groups (Figure 2) with a dose escalation between Group 1 (lower dose iNTS-GMMA vaccine, 10.6 µg total OAg content) and Group 2 (full dose iNTS-GMMA vaccine, 40 µg total OAg content). These first two groups will each consist of six participants who will be randomised 1:1 to the active vaccine or placebo. An additional six participants may be recruited to each of these groups if further safety information is required. Group 3 consists of 18 participants randomised 2:1 to receive the iNTS-GMMA vaccine or placebo. The decision to proceed to low or full dose vaccine in Group 3 will be based on safety reviews of Groups 1 and 2. There will be external safety monitoring reviews by the Data Safety Monitoring Committee (DSMC) between the two dose escalation groups and at a further two time-points in group three.

Figure 1 SALVO study design

Randomisation

Randomisation of participants will be carried out by unblinded study staff who are independent from the blinded team and do not perform any post vaccination procedures (such as ongoing eligibility or safety review). A web-based randomisation system will be used.

Blinding

This study will be conducted observer- and participant-blind from the time of randomisation until participant unblinding which will occur once the last participant has completed their final visit. Observer and participant blinding is required to minimise the risk of bias on the reporting of adverse events following the administration of vaccine.

Study visits

Vaccine or placebo will be administered at 0, 2 and 6 months. Participants will be directly observed for a minimum of 60 minutes following vaccination and then asked to complete an e- diary of their symptoms daily for 7 days following each vaccination. An in-person post-vaccination review will occur 7 and 28 days following each vaccine when participants will be reviewed for any possible adverse events. There are a total of 12 scheduled study visits and participants will be followed up for 1 year following first vaccination.

Recruitment and eligibility

Potential participants may be contacted by media advertisements, direct mail out or social media using an approved invitation letter or other approved advertising material to invite them to participate in the study. Participants will be reimbursed for their time, travel, and inconvenience. Healthy adults between the ages 18-55 years inclusive will be eligible for enrolment. Individuals will be initially screened for eligibility by telephone followed by face-to-face visits at the trial centre. Screening visits will involve obtaining informed consent (See SALVO Informed Consent Form in Supplementary Material 2), application of inclusion and exclusion criteria (summarised in Table 2) and clinical eligibility assessments including vital signs, physical examination, baseline blood tests, and urinalysis (SALVO Protocol in Supplementary Material 1).

Table 2 Summary of SALVO inclusion and exclusion criteria

Inclusion criteria	Willing and able to give informed consent for participation in the study
	Aged between 18-55 years inclusive
	In good health as determined by: - Medical history - Physical examination - Laboratory assessment - Clinical judgement of the investigators
	Willing to use highly effective contraception from one month prior to receiving the first vaccine and for the duration of the study (Females)
	Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
	Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study
	Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS)
	History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators.
	Have any known or suspected impairment or alteration of immune function.
	Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing

Exclusion criteria	Prior history of receipt of an oral typhoid vaccine (e.g.: Ty21a) within the last three years or a paratyphoid vaccine (as part of a clinical trial)
	Prior history of participation in a Typhoid or Paratyphoid controlled human infection study*
	Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
	Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (except for COVID-19 vaccines)
	History of allergy or anaphylaxis to a previous vaccine or vaccine components
	Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
	Participation in another research study involving an investigational product or that which may compromise the integrity of the study
	Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
	Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study
	Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m2
	Any other significant disease or disorder which, in the opinion of the Investigator, may: - Put the participants at risk because of participation in the study - Influence the result of the study - Impair the participant's ability to participate in the study

*Future studies in Typhoid or Paratyphoid endemic countries may consider including a past history of *S.* Typhi or *S.* Paratyphi natural infection as an exclusion. This was not included in this study as the risk of prior natural infection was considered low.

Sample Size and Statistical Analysis

The sample size in this study is 30-42 participants to account for additional participants to be recruited on DSMC advice. As the initial phase 1 trial primary objective is safety and tolerability this sample size has been chosen to evaluate early data on adverse events associated with increasing dose-level, with a larger subsequent phase 1 trial in a highly endemic country planned dependant on the trial safety data. The statistics for the primary endpoint are descriptive, with no testing of statistical significance. The confidence intervals will be set at 95%.

Ethics and Dissemination

As a first-in-human vaccine trial the iNTS-GMMA vaccine has undergone appropriate pre-clinical toxicology studies indicating a well-tolerated vaccine. Participants will be actively monitored for their safety during the trial by review of an electronic diary, visits, clinical observations, and safety blood tests and will have access to a 24-hour medical contact number. Appropriate risk and benefits of the study will be communicated to the participants, and informed consent will be taken prior to any study related procedures. Local and national guidelines on confidentially and data protection will be adhered to.

The DSMC consisting of an experienced group of clinicians and a statistician will be appointed to provide real-time independent oversight of safety and trial conduct. The DSMC will review safety data collated from participant and clinician recorded entries including

solicited and unsolicited adverse events, laboratory results and vital signs. Progression of enrolment from Group 1 to Group 2 to Group 3 will only occur after DSMC review of the relevant safety data. Further DSMC reviews will occur regularly throughout the trial. A development safety update report (DSUR) for the IMP will be prepared annually, on the anniversary of the MHRA approval for the trial.

Undertaking non-COVID-19 research during a dynamic COVID-19 pandemic represents a major logistical challenge. The safety of participants remains paramount and good infection prevention and control practices will be followed throughout the trial. The SALVO study team will monitor cases of COVID-19 within the participant cohort and the local population and will follow current national guidelines on COVID-19 with options including switching to phone appointments, halting, or extending the trial.

Once the trial has been completed including analysis of data, results will be published in a peer-reviewed journal and presented at conferences. The results of this study will directly impact the appropriateness of subsequent trials with a larger sample size to begin in a sub-Saharan country of high iNTS endemicity.

This study has been approved by South Central - Oxford A Research Ethics Committee on 28th April 2022.

Patient and public involvement statement

The protocol, study information booklet and recruitment materials were reviewed by a local patient consultation group who provided feedback and comments on the initial documents. Their comments led to changes in the participant-facing documents, ensuring they are easy and clear for participants to understand (Please see SALVO Participant Information Sheet in Supplementary Material 3).

Study Dates

Study recruitment began in May 2022. The estimated last participant last visit (LPLV) will be in December 2023.

Discussion

This will be the first phase 1 study investigating the iNTS-GMMA vaccine. The data generated by this trial will guide future vaccine development using this GMMA technology and may contribute to the licensure of the first vaccine against invasive non-typhoidal *Salmonella* species.

iNTS disproportionately causes severe disease in children under the age of 5 years in sub-Saharan Africa, and an effective vaccine will be of greatest benefit in this vulnerable population. As the UK is a country with a low burden of non-typhoid *Salmonella* disease (22, 23) healthy adult volunteers recruited to the SALVO study will be unlikely to have pre-existing immunity to *S*. Typhimurium or *S*. Enteritidis. However, assessment of the

immunogenicity of the iNTS-GMMA vaccine in an immunologically naïve cohort in SALVO will inform the decision to progress to a second larger phase 1 trial in a sub-Saharan African adult population where iNTS is endemic. This second study may provide insight into the vaccine-induced immunogenicity following pre-existing immunity in adults. Future studies will recruit African infants, in whom any future licensed vaccine is likely to be deployed. However without a known immune correlate of protection, the efficacy of the vaccine in prevention of iNTS disease cannot be determined without much larger field studies in endemic settings.

As GMMAs originate from the bacterial outer membrane they contain both the immunodominant OAg as well as multiple membrane proteins in their native conformation(19). Alongside measurement of OAg binding antibody after vaccination, this study will use functional antibody assays (including serum bactericidal activity), to interrogate the outer membrane protein specific responses. This may reveal conserved proteins present across multiple *Salmonella* serovars capable of eliciting pan-protective immune responses. As a first-in-human phase I trial, the study design prioritises the safety of the participants. The dose escalation design allows investigation of the safety of the vaccine in a small cohort and at a significantly lower dose prior to escalation to the full dose vaccine. Multiple DSMC and internal safety reviews at set intervals provide further mandatory time points to formally review the safety data in addition to real-time monitoring by the study team. This is a participant-observer blind, randomised, placebo-controlled study. Blinding of the trial aims to reduce both recall and observational bias, which is intended to allow confidence in the study's final outcome. The use of a placebo-controlled study design allows a direct comparison between active vaccine and placebo groups aiming to account for potentially confounding factors, such as the incidence of SAR-Cov-2 infection during the study.

SALVO will be the first clinical trial of the iNTS-GMMA vaccine. Currently, the only other iNTS-based vaccines to enter clinical trials are two trivalent vaccines covering *S. enterica* serovars Typhimurium, Enteritidis and Typhi (24, 25), with results to be published. A live attenuated oral *S.* Typhimurium was trialled in participants in 2009 but has not progressed further in the intervening years (26). Further iNTS-based vaccines are progressing through the pre-clinical phase including flagellin and OmpD-based vaccines (27).

The study protocol was prepared in accordance with the SPIRIT 2013 Checklist (28).

Authors' contributions

BH and MR designed and authored the protocol. BH and NK wrote the manuscript. NO, CF, HR, RW, PF, UN, RC, AJP contributed to the protocol design and/or study set up.

Conflicts of Interests

The iNTS-GMMA vaccine has been provided by the GSK Vaccines Institute for Global Health (GVGH). GVGH has reviewed the protocol developed by the Oxford Vaccine Group, University of Oxford and provided funding for clinical trial monitoring.

PF, UN and RC were employees of the GSK Vaccines Institute for Global Health at the time in which the study was conducted. UN owns shares in GSK. GSK Vaccines Institute for Global Health Srl is an affiliate of GlaxoSmithKline Biologicals SA. This does not alter the authors' adherence to all Journal policies on data and material sharing.

Funding

This research was funded in whole or in part by EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant. For the purpose of Open Access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission.

Data Statement

The Protocol Version 3.1 January 2023 has been published in supplementary material 1.

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Word Count 2636

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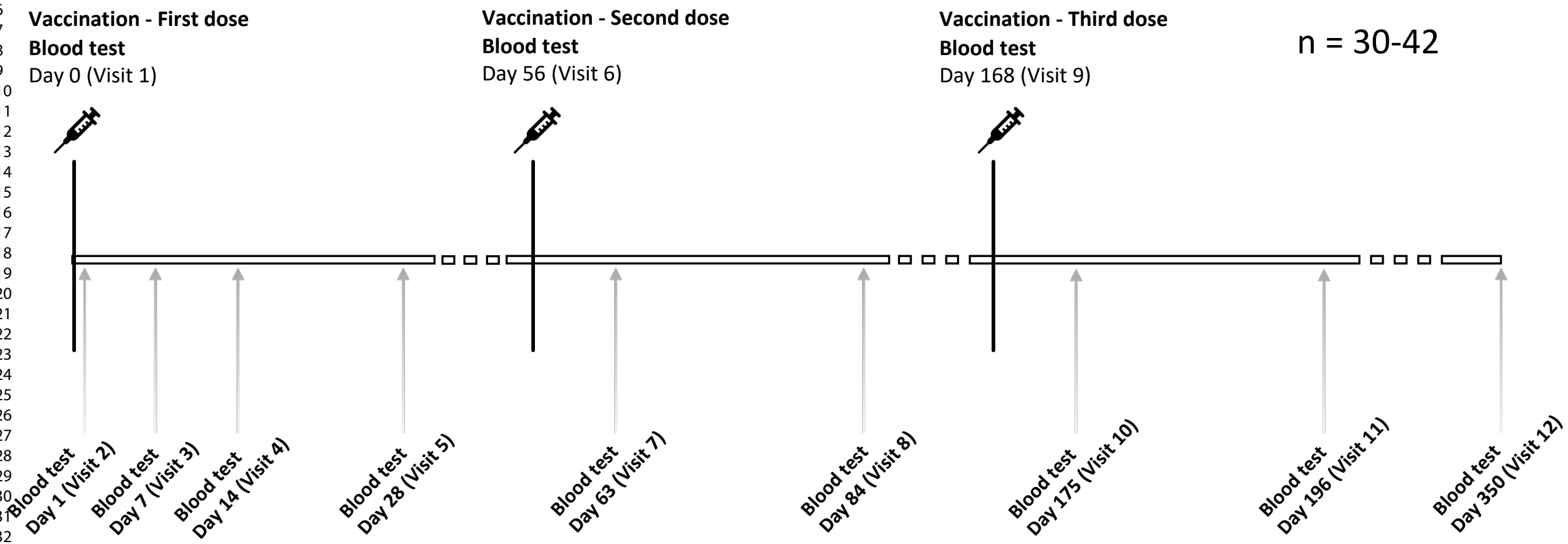
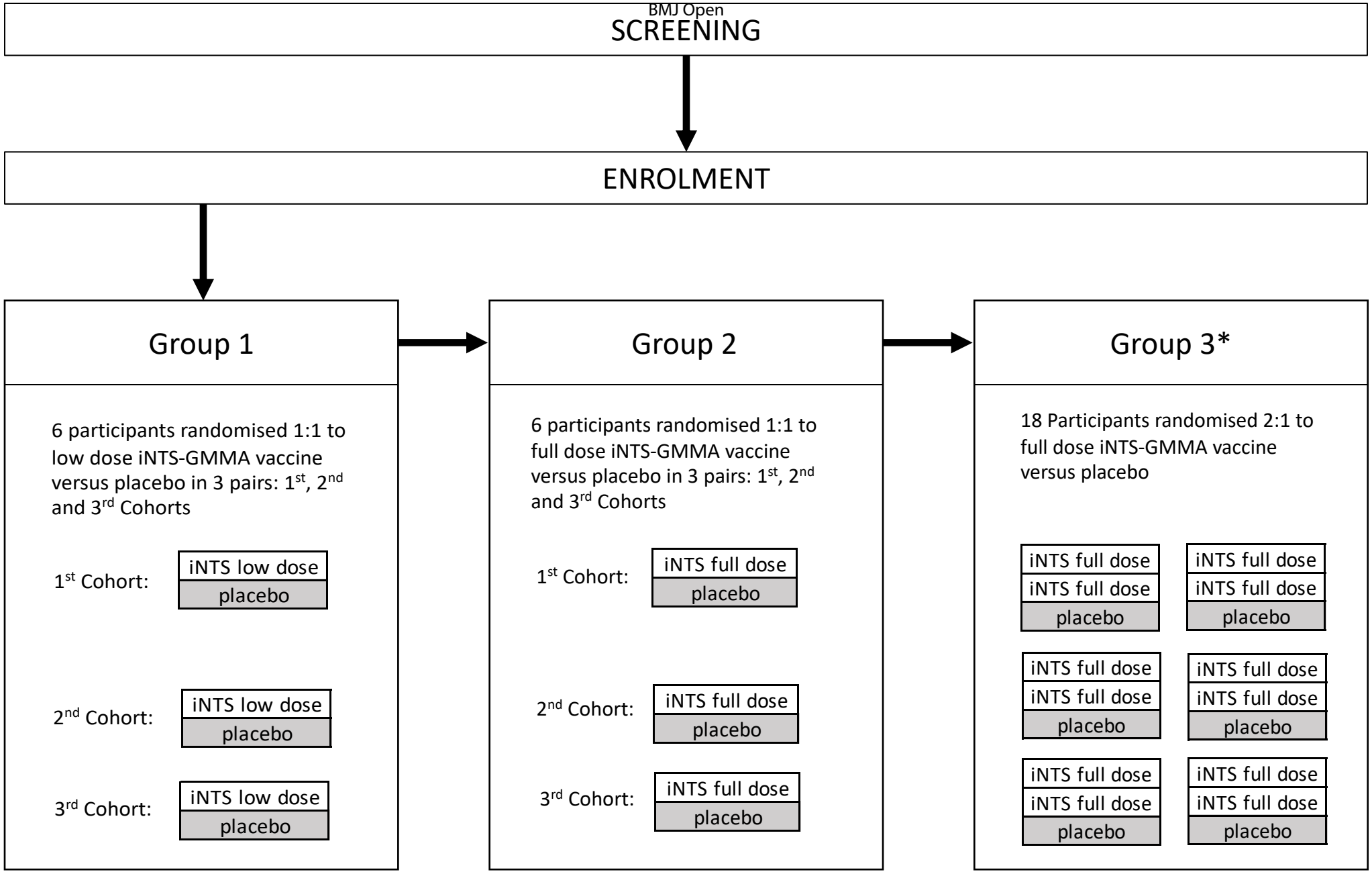

Vaccination - First dose
Blood test
Day 0 (Visit 1)
Vaccination - Second dose
Blood test
Day 56 (Visit 6)
Vaccination - Third dose
Blood test
Day 168 (Visit 9)
n = 30-42
Blood test Day 1 (Visit 2)
Blood test Day 7 (Visit 3)
Blood test Day 14 (Visit 4)
Blood test Day 28 (Visit 5)
Blood test Day 63 (Visit 7)
Blood test Day 84 (Visit 8)
Blood test Day 175 (Visit 10)
Blood test Day 196 (Visit 11)
Blood test Day 350 (Visit 12)



**Decision to proceed to low or full dose will be based on safety reviews of Groups 1 and 2.*

Trial Title: A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella

Internal Reference Number / Short title: OVG2020/01 Salmonella **V**accine Study in **O**xford (SALVO)
Ethics Ref: 22/SC/0059
IRAS Project ID: 1005098
EudraCT Number: 2020-000510-14
Date and Version No: 13 January 2023 / Version 3.1

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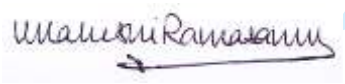
Sponsor: University of Oxford
Funder: EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS
The GSK Vaccines Institute for Global Health (Monitoring Costs Only)

Chief Investigator Signature:



Statistician Signature:

We declare no financial conflict of interest in this study.

Confidentiality Statement
This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

TABLE OF CONTENTS

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2 LAY SUMMARY

Nontyphoidal Salmonellae are types of bacteria that can cause gut infections resulting in diarrhoea, both in the UK and globally. However, under some circumstances, these bacteria can cause a more severe illness where infection spreads beyond the gut into the blood stream, a condition termed invasive non-typhoidal Salmonellosis (iNTS). iNTS disease is an under-recognised cause of disease and death in Sub Saharan Africa. In these regions, it primarily occurs in young children, particularly those with malaria and malnutrition. High death rates, difficulties in diagnosing this infection in the developing world, increasing resistance of the bacteria to common antibiotics, and spread via contaminated food and water make development of an effective and affordable vaccine against iNTS an essential control measure.

A new and innovative vaccine (iNTS-GMMA), has been developed which is based on the formation of bacterial outer surface particles. This vaccine facilitates exposure of components of the bacteria to the human immune system without the risk of causing infection. Developed by GSK Biologicals and GSK Vaccines Institute for Global health (GVGH), the aim of this vaccine is to confer immune protection to the most common African strains of the bacteria causing iNTS disease.

This study is a first-in-human clinical trial involving 30-42 healthy adult participants who will be randomly allocated to receive either iNTS-GMMA or a placebo. The main objective of this trial is to evaluate the safety of the iNTS-GMMA vaccine in healthy adults in the UK. The secondary objective is to investigate the human immune response to iNTS-GMMA vaccine.

3 SYNOPSIS

Trial Title	**A Phase 1 Clinical Study to Determine the Safety and Immunogenicity of a Novel GMMA Vaccine Against Invasive Non-Typhoid Salmonella**
Internal ref. no. (or short title)	**OVG2020/01 Sal**monella **V**accine Study in **O**xford (SALVO)
Trial registration	ISRCTN51750695
Sponsor	University of Oxford Research Governance, Ethics and Assurance Joint Research Office Boundary Brook House Churchill Drive Headington Oxford OX3 7GB United Kingdom
Funder	EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS The GSK Vaccines Institute for Global Health (Monitoring Costs Only)
Clinical Phase	Phase I, First in Humans
Trial Design	Single centre, participant-observer blind, randomised placebo-controlled safety and immunogenicity interventional study
Trial Participants	Healthy adults, aged 18-55 years inclusive
Sample Size	• 30-42 participants. • Up to 12 participants randomised 1:1 to receive lower dose at 10.6µg (3.8 dilution of full dose) OAg of iNTS GMMA vaccine or a placebo. • Up to 12 participants randomised 1:1 to receive the full dose at 40µg OAg of iNTS GMMA vaccine or a placebo • Eighteen participants randomised 2:1 to receive either the lower dose or full dose dependant on DSMC review versus placebo.
Planned Trial Period	• Total trial period including data analysis is 36 months. • The intended duration of a participant on the trial is 12 months. • The total duration from first participants first visit to last participants last visit is maximum 18 months.
Primary Objective	To determine the safety and tolerability between two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each

	adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years inclusive when given three doses of vaccine at 0, 2- and 6-month intervals.
Secondary Objectives	To investigate the immunogenicity at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Exploratory Objectives	To further investigate the immunogenicity using exploratory immunological analyses at two dose levels: • a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5) • a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5); in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.
Primary endpoint	The recording and assessment of local and systemic adverse events following administration of each vaccine dose: 1. Tenderness and pain at the injection site 2. Induration 3. Redness 4. Swelling 5. Headache 6. Malaise 7. Myalgia 8. Nausea and/or vomiting 9. Diarrhoea 10. Abdominal Pain 11. Anorexia 12. Arthralgia 13. Fatigue 14. Fever 15. Blood parameters (haematology / biochemistry) 16. Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR
Secondary endpoints	Immunological assays to study the immune responses to vaccines, including:

	1. Antibody concentration against serovar specific O antigen determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
Exploratory endpoints	Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to: 1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose. 2. Serum bactericidal antibody (SBA) titre against vaccine homologous strains before and after each dose 3. Serum bactericidal antibody (SBA) titre against a panel of other strains before and after each dose 4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose 5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose 6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose 7. Investigate the innate and adaptive response to the iNTS-GMMA vaccine by utilising next generation sequencing of the transcriptome to evaluate the differential gene expression profile and DNA storage for investigation of the genetic associations with the immune response 8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose 9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays 10. Faecal antibody concentration against O antigen determined by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection. 11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.
Investigational Medicinal Products	1. iNTS GMMA vaccine 2. Comparator (Placebo)
Form of vaccine	Glass vials containing 0.7ml sterile suspension containing either STmGMMA (80 µg/mL in OAg) or SEnGMMA (80 µg/mL in OAg) formulated with Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5

Form of Comparator (Placebo)	Glass vials containing 0.7ml sterile suspension containing Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH6.5
Dose	Lower Dose: 5.3 µg (OAg) STmGMMA/Alhydrogel + 5.3 µg (OAg) SEnGMMA/Alhydrogel (3.8x dilution of full dose generated by combining 0.5 mL of the two vaccine components into an empty vial and transferring 0.25 mL of the two components into 0.7 mL of the placebo vial, 0.5mL of combined vaccine to be administered) Full Dose: 20 µg (OAg) STmGMMA/Alhydrogel + 20 µg (OAg) SEnGMMA/Alhydrogel (generated by combining equal volumes of the two vaccine components in an empty vial, 0.5 mL of combined vaccine to be administered) Comparator (Placebo): 0.5mL to be administered
Route	Intramuscular
Vaccine Schedule	3 doses given at 0, 2 and 6 months

4 ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CCVTM	Centre for Vaccinology & Tropical Medicine
CI	Chief Investigator
CRA	Clinical Research Associate (Monitor)
CRF	Case Report Form
CT	Clinical Trials
CTA	Clinical Trials Authorisation
DMSC	Data Monitoring and Safety Committee
DSUR	Development Safety Update Report
ELISA	Enzyme Linked Immunosorbent Assay
GCP	Good Clinical Practice
GMMA	Outer membrane exosome from genetically modified Gram negative bacteria used as an antigen delivery system; GMMA is a pun on the Italian word for jewel or bud.
GP	General Practitioner
GVGH	GSK Vaccines Institute for Global health
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
iNTS	Invasive Non-typhoidal Salmonella
IRB	Independent Review Board
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
OVG	Oxford Vaccine Group
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PBMC	Peripheral Blood Mononuclear Cell
RGEA	Research Governance, Ethics and Assurance

R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SEn	*Salmonella* Enteritidis
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
STm	*Salmonella* Typhimurium
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TOPS	The Over volunteering Prevention System (http://www.tops.org.uk)

5 BACKGROUND AND RATIONALE

5.1 INVASIVE NON-TYPHOIDAL SALMONELLA DISEASE

Salmonella enterica is a rod-shaped Gram-negative bacterium that is further classified into approximately 2500 serovars, a number of which can cause human infection. Of these, *Salmonella* Typhi and Paratyphi are the causative agents of enteric fever and together are referred to as Typhoidal Salmonella. Non-typhoidal Salmonellae (NTS), such as *S.* Enteritidis and *S.* Typhimurium most commonly cause a self-limiting gastroenteritis that is indistinguishable from that caused by many other enteric pathogens. However, these organisms can also cause an invasive syndrome with bacteraemia, high fevers and metastatic infection which if untreated can lead to septicaemia and death. Invasive non typhoidal *Salmonella* (iNTS) infections are more common in resource poor settings of sub-Saharan Africa, children, the elderly and in the immunosuppressed, including HIV-infected individuals. Unlike *S.* Typhi and *S.* Paratyphi, whose only reservoir is humans, NTS can be acquired from multiple animal reservoirs including domestically farmed animals[1–3]. However, data from sub-saharan Africa appears to suggest that human-to-human transmission remains the primary mode of dissemination[4].

5.2 BURDEN OF DISEASE

The Global Burden of Disease Study estimates 535,000 annual global cases of iNTS; associated with 77,500 deaths in 2017 alone, representing a higher case fatality rate when compared with non-typhoid *Salmonella* gastroenteritis or typhoidal *Salmonella*[5].

In the UK, NTS infection is a frequent cause of foodborne gastroenteritis outbreaks. Public Health England surveillance data showed that NTS was isolated from 8630 patient samples in 2016[6]. Whilst there are no human seroprevalence studies, it is clear that NTS remains endemic; particularly in livestock and poultry, with multiple veterinary programs implementing control methods such as testing and vaccination[7]. Although less common than in other parts of the world, invasive syndromes do also occur in the UK. Between 2004-2015, there were 2484 iNTS blood isolates in England, with neonates, individuals aged over 65 and men more likely to have a bacteraemia[8]. A case series of 82 iNTS blood cultures at a single site in the UK, found bacteraemias without an intestinal focus to be related to underlying immunosuppression in 80% of cases[9].

By far the highest burden of iNTS disease throughout the world remains in sub-Saharan Africa with 78.8% of global cases[5]. However, this is a likely underestimate of the true burden of disease, given the limited availability of diagnostics (blood cultures) in this setting. In addition, the clinical presentation of invasive disease in children is poorly defined, typically presenting as a febrile illness similar to malaria, enteric fever or pneumonia. Nevertheless, iNTS remains one of the most commonly identified causes of bacteraemia in the region[3]. A study in Malawi focused on adult medical admissions found iNTS to be the most common isolate from blood cultures, contributing to 37% of all bacteraemias in a 12 month period, and associated with a 33% case fatality rate[10]. A study in Ghana in a paediatric population found that 23.5% of bacteraemias were associated with *Salmonella* sp. of which 59% were associated with *Salmonella* Enteritidis[11]. Another paediatric single-centre study in Malawi found a case fatality rate of 20% in invasive *Salmonella* disease, of which 94% was associated with iNTS. The highest incidence was in the under three age group with 81% of cases, with a median age of 16 months[12].

The age at which infection occurs show a bimodal distribution in most African studies[2,13]. The majority of infections are in the under 5 age group with 68.3% of cases[13]; with a second peak in 30-40 years age group, which is believed to be associated with the higher incidence of HIV, malaria and malnutrition[2,13]. The Global Burden of Disease estimates a disproportionably high incidence of approximately 233,400 cases, including 31,630 deaths in the under 5 year olds globally in 2017[5].

In a meta-analysis of 22 African studies of all ages, *Salmonella* sp. was the most prevalent isolate in blood cultures at 29.4 %, with iNTS accounting for 58.4%. Of those patients who had additional HIV testing, iNTS was particularly associated with HIV infection (OR 8.2) compared with *Salmonella* Typhi bacteraemia (OR 0.07)[14]. In addition, studies have shown a decline in iNTS to be associated with a reduction in HIV, as a result of the availability of ART, as well as public health measures designed to minimise the impact of malaria and malnutrition[3,15,16]. *Salmonella* Enteritidis (SEn) and *Salmonella* Typhimurium (STm) represent the most common serovars to cause invasive disease both globally and within sub-Saharan Africa. *Salmonella* Typhimurium isolates with the multi-locus sequence type (MLST) ST313 have emerged as a dominant subtype in sub-Saharan Africa. Genomic analysis of these isolates reveals genomic degradation and pseudogene formation, characteristics consistent with host adaptation and restriction by the bacterium. Along with enhanced invasive and virulence factors, the ST313 subtype is associated with multidrug resistance, potentially contributing to higher incidence and mortality of iNTS in sub-Saharan Africa[3,17]. In contrast, UK isolates are usually MLST ST19 and are commonly associated with gastroenteritis.

5.3 IMMUNO-PATHOGENESIS

It remains unclear why some infections result in local self-limiting disease whilst others cause invasive disease. In adults, iNTS is more common in the immunocompromised, including individuals with HIV, malaria, malnutrition, sickle cell disease and other immunodeficiency states, such as chronic granulomatous disease[2,3].

In children under 5 years of age, acquisition of functional antibody is associated with protection from *Salmonella* infection. Thus the highest peak of infection is between 6 months to 2 years of age, with the incidence declining thereafter. This observation correlates with protection from placentally transferred maternal antibody waning by 6 months and the development of detectable functional antibodies from natural exposure by 16 months of age[18,19]. A study in Malawi found detectable functional antibodies peaked at 35 months. In addition, this study found STm-specific CD4 T helper cells peaked at 14 months then declined, suggesting CD4 T helper cells alone were not protective.

Functional antibodies targeting iNTS can effect bacterial killing though activation of the classical pathway of complement and subsequent membrane attack complex assembly or by opsonisation, facilitating phagocytosis and oxidative burst-mediated intracellular killing. Possible targets for functional antibody include the O antigen (OAg) component of outer membrane lipopolysaccharide (LPS) or outer membrane protein antigens (OMP Ag) or flagella antigen (FliC). Human studies suggest that bactericidal activity correlated with the detectable levels of IgG to Lipopolysaccharide (LPS) but not to outer membrane protein (OMP) or flagella proteins. However antibodies against the FliC flagellar protein and membrane-bound porin proteins such as OmpD have been shown to be protective in mouse models[20,21].

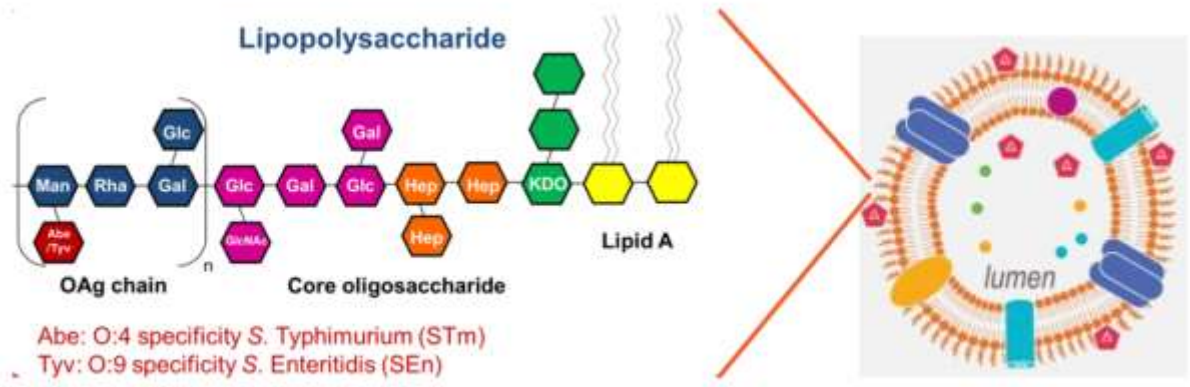


Figure 1.1 Diagram of O Antigen and Lipopolysaccharide constituent of GMMA (taken from Koeberling et al., 2017[22])

Together with lipid A, OAg forms part of the lipopolysaccharide attached to the outer membrane of Salmonella. OAg is the most immunodominant part of LPS and functions as a virulence factor with multiple studies showing attenuation of virulence in bacteria with mutations impairing OAg synthesis and longer OAg chains associated with increased resistance to antibody and complement mediated killing[23]. OAg structure can vary dependant on the individual oligosaccharide units, and this forms the basis of serotyping commonly used in microbiology laboratories. STm is associated with O:4,5 whilst SEn is associated with O:9[24].

5.4 PRIOR EXPERIENCE WITH iNTS VACCINES

The WT05 vaccine produced by Microscience Ltd. is the only iNTS vaccine candidate to date that has progressed to a phase one clinical trial. This dose escalation study randomised participants to receive either an oral attenuated *Salmonella* Typhi or an oral attenuated *Salmonella* Typhimurium at a dose 10^7, 10^8 or 10^9 CFU. Both strains were attenuated with mutations in aroC and ssaV, with ssaV associated with a reduction in function of the type III secretion system. Of the nine participants who received the *Salmonella* Typhimurium oral vaccine only the three participants in the 10^9 group had a significant antibody response[25]. There were no serious adverse events observed and each dose level of oral attenuated Salmonella *Typhimurium* appeared well tolerated. Other vaccines in development include an oral live attenuated vaccine (CVD 1931, CVD 1994), a bivalent OAg conjugate vaccine (COPS, flagellin) and protein vaccines (flagella and OmpD). All remain in the pre-clinical phase[26,27].

5.5 RATIONALE FOR VACCINE BASED ON GMMA TECHNOLOGY

The outer membrane of Gram-negative bacteria such as *Salmonella* naturally release outer membrane vesicles (OMVs) containing outer membrane proteins. OMVs have clear potential as vaccines, displaying immunogenic surface antigens in their natural conformation whilst avoiding the risks of potential infection associated with the use of live attenuated vaccines. Detergent-extracted OMVs from homogenised bacteria have been successfully licensed and used as vaccines against capsular group B meningococcal infections (MenBvac, Bexsero, VA-MENGOC-BC).

In contrast to traditional OMVs, the GMMA-technology derives outer membrane exosomes by induction of hyper blebbing from viable genetically modified bacteria. iNTS GMMA producing

vaccine strains are created from wild-type strains by deletion of *tolR*, *msbB* and *pagP* genes from *S.* Typhimurium and *S.* Enteritidis. Together, these modifications facilitate increased production of membrane blebs (Δ*tolR*) and reduced-acylation of the lipid A component of bacterial lipopolysaccharide (LPS via Δ*msbB* and Δ*pagP*). GMMA particles with hexa- or penta-acylated LPS induce less cytokine production *in vitro* by human peripheral blood monocytes compared to wild type GMMAs (containing hepta-acyltaled lipid A), which potentially reduces *in vivo* reactogenicity. Furthermore, GMMAs are potentially highly immunogenic, as they present O polysaccharide (OAg) and outer membrane protein antigens identified as immune targets in their native configuration, with minimal expression of cytoplasmic or inner membrane proteins. Purified GMMA particles are filtered, concentrated and implemented as a vaccine[28,29].

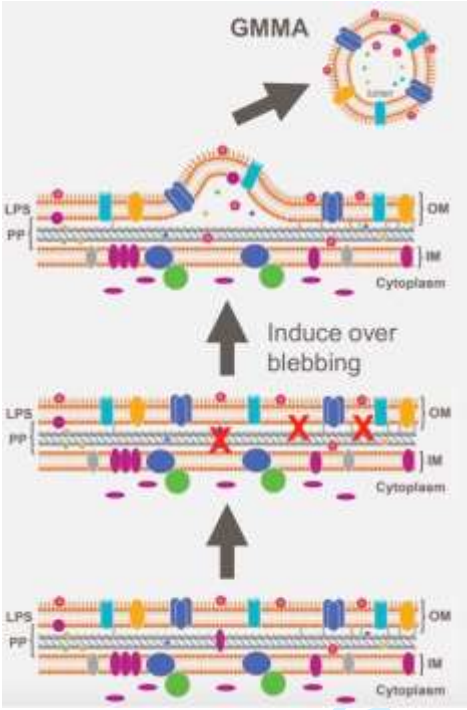


Fig 1.2 Outer membrane blebbing to create GMMA particles (taken from Koeberling 2017[22])

5.6 CLINICAL STUDIES OF ENTERIC GMMA VACCINES

This is the first trial to investigate a 2-component iNTS GMMA vaccine in humans. However, there have been four proof-of-concept clinical trials of a *Shigella* Sonnei GMMA vaccine; three phase 1 trials (including one phase 1 extension trial) in Europe[30,31] and one phase 2a trial in Kenya[32]. Over 100 participants have been enrolled, with all studies demonstrating a well-tolerated vaccine with an acceptable safety profile. The most common adverse event was mild to moderate injection site pain. A transient asymptomatic neutropenia was reported in 9.6% of participants in the two initial phase 1 European trials[30].

In vitro analysis of *Shigella* GMMA have revealed they contain > 95% of the outer membrane and periplasmic proteins[33]. By extrapolation this suggests the iNTS GMMA vaccine may contain a similar percentage of proteins which may contain multiple antigens including OAg and OMP Ag eliciting a potentially broadly protective immune response.

In Phase I trials, the *Shigella* GMMA vaccine used a 3-dose regime with intervals of 0, 1 and 2 months. The iNTS GMMA vaccine will use a 3-dose regimen at 0, 2 and 6 months to allow involution of B cell germinal centres and T cell responses between doses to better investigate

booster and memory responses. The highest *Shigella* GMMA vaccine dose so far tested contained approximately 6 µg OAg and 100 µg protein, while the highest iNTS GMMA dose will contain 40 µg OAg and approximately 31 µg protein.

THE INVESTIGATIONAL PRODUCT: 2-COMPONENT iNTS GMMA SALMONELLA VACCINE

5.7 DESCRIPTION OF INVESTIGATIONAL PRODUCT

The iNTS GMMA Vaccine consists of the outer membrane exosomes of the two most common serovars causing invasive disease, *Salmonella* Enteritidis (O:9) and S*almonella* Typhimurium (O:4.5), adsorbed to Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline pH 6.5. Two doses levels will be used for this study: a full dose of 20 µg STmGMMA as OAg and 20 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml full dose; and a lower dose at 3.8 dilution rendering a vaccine consisting of 5.3 µg STmGMMA as OAg and 5.3 µg SEnGMMA as OAg on 0.35 mg AL^{3+} / 0.5 ml dose. The placebo matches the vaccine matrix and consists of Alhydrogel (0.35 mg AL^{3+} / 0.5 mL dose) in isotonic 20mM Phosphate buffered saline pH 6.5 without a GMMA component. The vaccine and placebo are both administered as intra-muscular injections.

5.8 CHARACTERISTICS OF VACCINE PREPARATION

STmGMMA and SEnGMMA, the iNTS vaccine active components, are formulated separately at 80 µg GMMA as OAg/ml batches with the GMMA absorbed onto 0.7mg Alhydrogel as Al^{3+}/ml Alhydrogel and suspended in isotonic 20mM Phosphate buffered saline at pH6.5 . Each batch is aliquoted into vials containing 0.7 ml of either STmGMMA/ Alhydrogel or SEnGMMA Alhydrogel, stored at 2-8 ºC.

At the clinical site, 0.5ml of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel will be mixed in an empty vial using a standard operating procedure to yield 1ml of the 2-component iNTS GMMA vaccine containing 40 µg STmGMMA as OAg and 40 µg SEnGMMA as OAg and 0.7 mg Al^{3+} of Alhydrogel suspended in isotonic 20mM Phosphate buffered saline pH 6.5. This procedure including dilution for the lower dose iNT-GMMA vaccine is detailed in the clinical study plan and will be performed at the clinical site by trained study personnel. This procedure has been developed and evaluated by GVGH. Further stability testing has been performed to confirm the quality of the iNTS-GMMA vaccine for up to 6 hours post mixing, however it is envisaged that the final iNTS-GMMA vaccine will be used within 1 hour of mixing.

5.9 IMMUNOGENICITY IN MICE

Anti-OAg ELISA titres correlate with functional antibody assays consistent with immunity in children[18,19]. Mouse studies using challenge with either wild type *Salmonella* Typhimurium or with an OAg knockout strain higher levels of functional bactericidal antibodies in animals exposed to the bacteria expressing OAg [24].

Mice immunised either with STm and SEn GMMA vaccine showed both an elevated OAg titre by ELISA and complement mediated antibody killing via serum bactericidal assay (SBA). These results were then replicated in mice immunised with the two component (STm and SEn) iNTS GMMA vaccine (Figure 1.3) confirming the immunogenicity and validity of the bivalent

vaccine[34]. On challenge with either live *S.* Typhimurium or *S.* Enteritidis, immunised mice showed a reduction of bacterial burden (CFUs) in the spleen and liver.

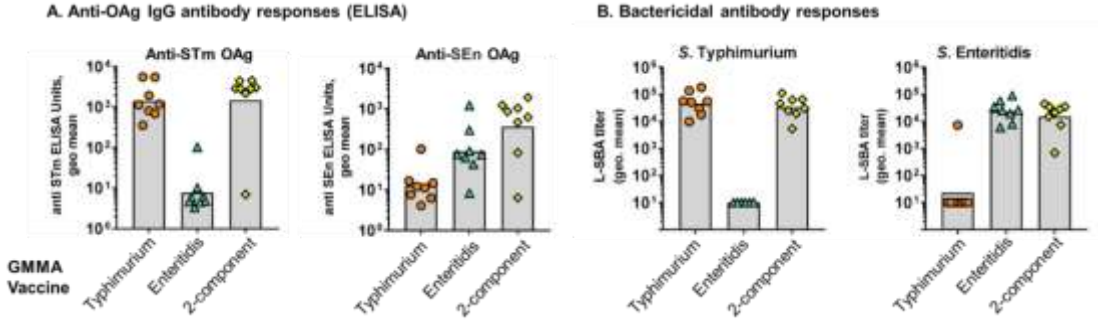


Figure 1.3 (A) Anti-STm and anti-SEn OAg IgG serum antibody responses (ELISA units) and (B) SBA responses against *S.* Typhimurium and *S.* Enteritidis strains. CD1 mice were immunized twice, four weeks apart with 0.16 µg (based on OAg) STmGMMA or SEnGMMA adsorbed on Alhydrogel or a mixture of 0.16 µg each of the two formulated GMMA (2-component iNTS-GMMA). Sera were collected two weeks after the second immunization. Symbols represent results from individual mice, bars represent group geometric mean titres.

In addition there was no significant difference in antibody response when the GMMAs were combined with Alhydrogel[34]

Study	Vaccine	Adsorbent	Dose [µg]	Anti-OAg IgG ELISA titre (geometric mean)
1	STmGMMA	None	2.5	61588
	STmGMMA	Alhydrogel	2.5	69572
2	SEnGMMA	None	2.5	8953
	SEnGMMA	Alhydrogel	2.5	10401

Table 1.1 Anti-OAg IgG ELISA units induced in mice by STmGMMA and SEnGMMA administered with or without Alhydrogel

5.10 IMMUNOGENICITY IN RABBITS

OAg is also a immune target in rabbits. Animals challenged with wild type *Salmonella* Typhimurium or OAg knockout strains develop higher bactericidal antibody response in the wild type arm[24].

In a rabbit immunogenicity study, STm GMMA/Alhydrogel, SEn GMMA/Alhydrogel and the 2-component iNTS-GMMA vaccine were well tolerated and induced strong anti-STm OAg and anti-Sen OAg serum IgG responses and high bactericidal antibody activity against *S.* Typhimurium and *S.* Enteritidis strains.

5.11 TOXICOLOGY

The dosing regimen has been estimated based on immunogenicity in mice and rabbits[24,34], in conjunction with the results of monocyte activation, pyrogenicity and repeat dose toxicology studies in rabbits.

In order to maintain immunogenicity whilst reducing reactogenicity, expression of Lipid A, a potent stimulator of the innate immune system, has been modified via deletions of the genes msbB and pagP. A monocyte activation model with human PBMC stimulated by a mixture of 1:1

STm and SEn iNTS GMMAs produced less IL-6 (a marker of inflammation) than wild-type GMMAs[24]. A similar cytokine release profile was stimulated by unformulated *S. sonnei* 1790-GMMA, which, as Alhydrogel formulated vaccine, were well tolerated in EU and Kenyan adults.

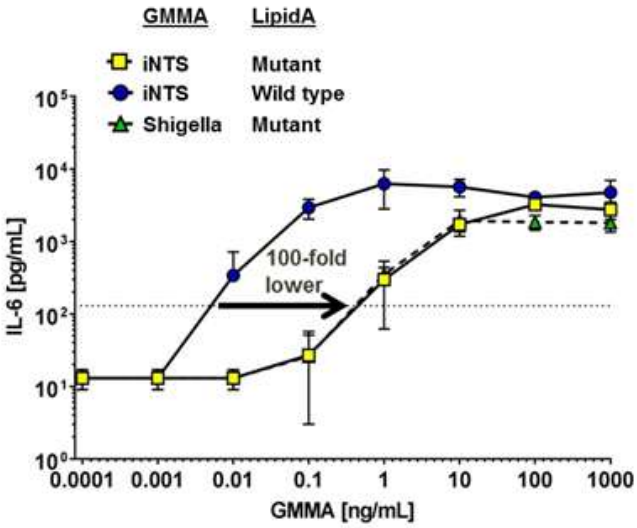


Figure 1.4 Monocyte activation test IL-6 response after incubation with different concentrations of a 1:1 mixture of STmGMMA and SEnGMMA in comparison to *S. sonnei* 1790-GMMA

A rabbit pyrogenicity study was performed using intramuscular administration of undiluted single GMMA formulations and 2-component iNTS-GMMA vaccine. The 2-component iNTS-GMMA vaccine showed a mean peak temperature rise of 1.1^{O}C using the highest target human iNTS dose. This mean peak temperature rise was 0.8 ^{O}C less than the mean peak temperature rise without Alhydrogel, suggesting whilst there is minimal change in immunogenicity, reactogenicity is reduced with the addition of Alhydrogel.

	Group mean initial temperature [°C]	Group mean maximum temperature [°C] with time indicated	Group mean temperature [°C] at end of measurement [5 hours after vaccination]	Mean peak temperature time point after vaccination [minutes]	Mean peak temperature rise [°C]
iNTS-GMMA (STmGMMA/Alhydrogel + SEnGMMA/Alhydrogel) 20 µg + 20 µg [OAg]	38.6	39.7	39.6	210	1.1
Unformulated mixture of STmGMMA + SEnGMMA 2 µg + 2 µg [OAg]	39.3	41.2	39.8	180	1.9

Table 1.2 STn+SEn with or without Alhydrogel dosed rabbits, showing a mean temperature rise of 1.1 ^{O}C in STm/Sen with Alhydrogel (iNTS Vaccine)

A repeat dose toxicology study in rabbits has been performed using the highest anticipated human iNTS dose at Covance Laboratories (Harrogate, UK).

Group number	Group description	Dose level (µg GMMA [OAg])	Dose Days	Animal numbers			
				Main necropsy		Recovery necropsy	
1	Control (Saline)	0	1, 15, 29, 43	5 Male	5 Female	4** Male	5 Female
2	Test	20 + 20*	1, 15, 29, 43	5 Male	5 Female	5 Male	5 Female
*The test article was 0.5 mL of a bed-side mixing of SEnGMMA/Alhydrogel and STmGMMA/Alhydrogel each containing 20 µg of each GMMA (quantified based on OAg) and 0.35 mg Al^{3+} Alhydrogel **One animal was withdrawn during the pre-immunization phase							
Main necropsy: Day 46 (3 days after last injection) Recovery necropsy: Day 71 (28 days after last injection)							

Table 1.3 Outline of repeat dose toxicology study in rabbits. INTS GMMA vaccine versus Saline Control Group

New Zealand White rabbits were grouped into a test and control (saline) group. They then received 0.5ml (40µg OAg) of the iNTS-GMMA vaccine at 2 weekly intervals. The results indicated a systemically well-tolerated iNTS GMMA vaccine. Please see the Investigator's Brochure for further details.

5.12 RATIONALE / AIM OF TRIAL

Thirty to forty-two participants in the 18-55 year age group will be recruited into the trial. The trial will follow a dose escalation design with the first group randomised 1:1 to receive a lower dose of 10.6 µg total OAg of iNTS-GMMA vaccine (5.3 µg of STmGMMA as OAg and 5.3 µg of SEnGMMA as OAg) or placebo. A second group will be randomised 1:1 to receive the full intended dose of 40 µg total OAg of iNTS-GMMA (20 µg of STmGMMA as OAg and 20 µg of SEnGMMA as OAg) or placebo. A third group will be randomised 2:1 to receive the iNTS-GMMA vaccine at intended full dose versus a placebo (See 7.Trial Design). An adequate safety review from the Data Safety Monitoring Committee (DMSC) will allow progression of the trial from group 1 to group 2 and from group 2 to group 3. Given there are no current licensed iNTS vaccines, the comparator for this trial will be an Alhydrogel placebo. The last dose of the vaccine will be on Day 168, with a final visit 6 months thereafter. Thus, participants will remain on the trial for 12 months in total.

The aim of the trial is to determine the safety of the iNTS-GMMA vaccine. The immunogenicity of the vaccine will be assessed by OAg ELISA and serum bactericidal assays specific to each vaccine serovar. Additional exploratory immunological assays assessing functional antibody (including but not limited to opsonophagocytosis and glycosylation), B cell and T cell responses will be performed. If the results of this trial indicate a vaccine with an adequate safety profile, progression to a further phase 1 clinical study in an iNTS endemic country will occur.

6 OBJECTIVES AND OUTCOME MEASURES

6.1 PRIMARY OBJECTIVE

To determine the safety and tolerability between two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.2 SECONDARY OBJECTIVE

To investigate the immunogenicity at two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals.

6.3 EXPLORATORY OBJECTIVES

To further investigate the immunogenicity using exploratory immunological analyses of the two dose levels:

- a lower dose of the iNTS-GMMA vaccine (5.3 µg STmGMMA in OAg and 5.3 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5)
- a full dose of the iNTS-GMMA vaccine (20 µg STmGMMA in OAg and 20 µg SEnGMMA in OAg, each adsorbed on 0.35mg AL^{3+} / dose in isotonic 20mM Phosphate buffered saline pH 6.5);

in healthy adults 18-55 years when given three doses of vaccine at 0, 2- and 6-month intervals

6.4 PRIMARY ENDPOINTS / OUTCOME MEASURES

The recording and assessment of local and systemic adverse events following administration of each vaccine dose;

- Tenderness and pain at the injection site
- Induration
- Redness

- Swelling
- Headache
- Malaise
- Myalgia
- Nausea and/or vomiting
- Diarrhoea
- Abdominal Pain
- Anorexia
- Arthralgia
- Fatigue
- Fever
- Blood parameters (haematology / biochemistry)
- Any unsolicited symptom(s) not listed above in addition to any other AE, SAE or SUSAR

6.5 SECONDARY ENDPOINTS / OUTCOME MEASURES

Immunological assays to study immune responses to vaccines, including:

1. Antibody concentration against serovar specific O antigens determined by enzyme linked immunosorbent assay (ELISA) before and after each dose.

6.6 EXPLORATORY ENDPOINTS / OUTCOME MEASURES

Exploratory Immunological assays to study the immune responses to vaccines, including but not limited to:

1. Antibody concentration against other potential antigens including porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose .
2. Serum bactericidal antibody (SBA) titres against vaccine homologous strains before and after each dose
3. Serum bactericidal antibody (SBA) titre isogenic strains before and after each dose
4. Functional antibody analyses which may include opsonophagocytic assays and glycosylation before and after each dose
5. Quantification of circulating vaccine-induced B-cells responses specific for vaccine antigens before and after each dose
6. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after each dose
7. Transcriptomic profile analysis after immunization to investigate differential expression of innate, B cell and T cell activation gene modules and DNA storage for investigation of the genetic associations with the immune response
8. Oral fluid antibody concentration against O antigen and porins determined by enzyme linked immunosorbent assay (ELISA) before and after each dose
9. Create a human reference serum standard against iNTS for set-up of laboratory antibody assays

10. Faecal antibody concentration against O antigen determined by by enzyme linked immunosorbent assay (ELISA) in a subset of participants who opt-in to stool sample collection.
11. To investigate a potential relationship between the composition of the gut microbiota and vaccination outcome in a subset of participants who opt-in to stool sample collection.

7 TRIAL DESIGN

7.1 OVERVIEW OF TRIAL DESIGN

This is a first in human, phase 1, single-centre participant-observer blind study to assess the safety and immunogenicity of three administrations of iNTS-GMMA vaccine in healthy adults. Participants will be considered enrolled in the study once their first vaccination has been administered. The total number of participants is 30-42, and individuals will be divided into 3 groups as described below:

1. **<u>Group 1</u> (PARTICIPANT-OBSERVER BLIND)** - lower dose 10.6 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or a placebo according to the study plan outlined in section 7.2.1. A favourable DSMC review of the safety data from this arm will be required before commencement of group 2. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

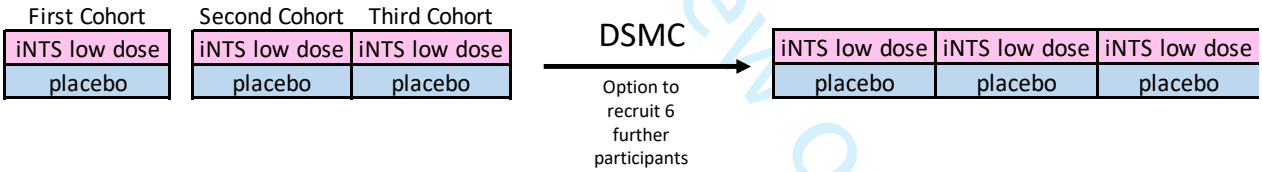


Figure 2.1 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive lower dose iNTS-GMMA vaccine or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

2. **<u>Group 2</u> (PARTICIPANT-OBSERVER BLIND)** - full dose 40 µg total OAg of iNTS-GMMA vaccine (three administrations at D0, D56 and D168). This group will only proceed after DSMC review and approval of Group 1. This group will consist of 6 participants subdivided into 3 cohort pairs. Within each pair the two participants will be randomised 1:1 to receive the 40 µg total OAg of iNTS GMMA vaccine or a placebo. See section 7.2.1 for further details regarding safety and dose escalation. A favourable DSMC review of the safety data from this arm will be required before the commencement of group 3. If the DSMC require further safety data at this dose level a further 6 participants subdivided into 3 cohort pairs may be enrolled.

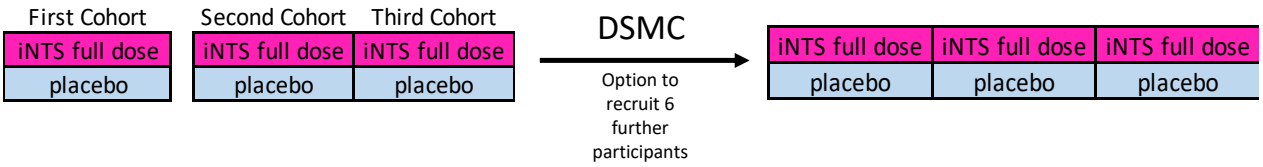


Figure 2.2 Six Participants, subdivided into three cohorts, each cohort consisting of two participants, randomised 1:1 to receive iNTS full dose or placebo; option to recruit further 6 participants (3 pairs) depending on DSMC review

3. **Group 3 (PARTICIPANT-OBSERVER BLIND)** – 18 participants will be randomised 2:1 to receive three administrations at D0, D56 and D168 of either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or a placebo. Recruitment to this group will be subject to a favourable interim review of the safety data of group 2 by the DSMC.

   Depending on DSMC review and participant numbers in Group 1 and 2, 18 participants will be enrolled into Group 3:
   Either:

iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose	iNTS full dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.3 Eighteen Participants, randomised 2:1 to receive full dose iNTS-GMMA vaccine or placebo

Or:

iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose	iNTS low dose
placebo	placebo	placebo	placebo	placebo	placebo

Figure 2.4 Eighteen Participants, randomised 2:1 to receive lower dose iNTS-GMMA vaccine or placebo

7.2 DOSE ESCALATION

As this is a first in human trial, we will operate a dose escalation policy in groups 1-3 between lower and full doses. The rationale for these doses is based on experience from animal toxicology studies (5.11 TOXICOLOGY). Escalation between these doses will be dependent on a favourable safety review by the DSMC. This unblinded safety review will consist of AEs from all participants vaccinated in group 1, 2 and 3 at the DSMC review time points indicated below. Group 1 and 2 must have a minimum of 6 participants vaccinated in each group prior to DSMC review. AE Safety Data will include solicited, unsolicited, observation related, laboratory, SAE / SUSARS. Note SAE/SUSARs have specific DSMC reporting instructions as outlined in Section 11.6.

7.2.1 DOSE ESCALATION PROCESS FOLLOWING D0 VACCINE

Group 1 – Lower dose IMP

Initially, 6 participants will be subdivided into 3 pairs. Within each pair the two participants will be randomised 1:1 to receive the 10.6 µg total OAg of iNTS-GMMA vaccine or placebo. Initially

only one pair will be vaccinated and observed for any adverse reactions for 48 hours before further participants are vaccinated. The medically qualified investigator will be asked to provide the decision on whether to proceed after a blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a second and third pair will be vaccinated at least one hour apart. This will bring the total number of participants in this group to 6, of which 3 receive the lower dose IMP, and 3 receive the placebo.

Group 1: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 1. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same lower dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 2 will proceed.

If participants in Group 1 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 2 – Full dose IMP

In the full dose group, 6 participants will initially be subdivided into 3 pairs as in group 1. Within each pair the two participants will be randomised 1:1 to receive the full dose 40 µg total OAg of iNTS-GMMA vaccine or placebo. Initially only one pair will be vaccinated and observed for any adverse reactions/events for 48 hours before more volunteers are vaccinated. The medically-qualified investigator will be asked to provide the decision on whether to proceed after blinded safety review (on the basis of participant clinical and e-diary reviews) of the first paired cohort. If there are no safety concerns then a further two paired cohorts will be vaccinated with the 40 µg total OAg dose. This will bring the total number of participants in this group to 6, of which 3 receive the full dose IMP, and 3 receive the placebo. The second and third pairs will be vaccinated at least one hour apart.

Group 2: DSMC (Unblinded) Review

A DSMC review will be triggered once at least 7 days of data are available for the first 6 participants in group 2. This review will include the assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Should further safety data be required the unblinded DSMC may request a further 6 individuals in 3 pairs to be vaccinated at the same full dose, with at least an hour between the vaccinations of each pair. Following a favourable review from the DSMC, enrolment and vaccination to the full dose group 3 will proceed.

If a favourable opinion is given for Group 1, but not for Group 2, then the DSMC may allow the ‘lower dose’ to be used in Group 3 instead of the full dose. If favourable decision is received

from the DSMC a substantial amendment would be required to continue further vaccination clearly stating the iNTS-GMMA vaccine dose to be administered.

If participants in Group 2 develop adverse events which meet the group holding rules as detailed in Section 11.14, the study will be paused, with no further vaccinations administered, pending DSMC review. If a favourable decision is received from the DSMC a substantial amendment would be required to continue further vaccination.

Group 3 – Low or Full Dose

In this group, 18 participants will be randomised 2:1 to receive the iNTS GMMA vaccine (either the lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo. The total number of participants in the study will be between 30-42 individuals, depending on the final numbers of participants recruited to group 1 and 2.

7.3 DSMC (Unblinded) REVIEWS

The DSMC will review the unblinded safety data at two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

7.4 GROUP ALLOCATION

Allocation to each group will be decided by order of enrolment into the trial. Participants will be considered enrolled in the trial once they receive their first vaccination. Groups 1, then 2 will be preferentially recruited to ensure study progress as per the dose escalation process above with randomisation within each cohort pair. Once safety data have been reviewed by the DSMC, participants recruited to group 3 will be randomised to receive either iNTS GMMA vaccine (either lower dose or full dose, depending on the safety results of Group 1 and 2) or placebo in a 2:1 ratio.

7.5 SAFETY MONITORING

Safety outcomes of the participants will be monitored throughout the study. This will be done by monitoring symptoms at visits, daily review of an electronic symptom diary up to D6 after every vaccine received on the study, and safety blood tests (Table 2.1).

Group 1 and 2 vaccinations will proceed in a staggered fashion (Fig 2.2), dependent on safety review by the DSMC. The time interval between the 7 days post the last participant to receive their first vaccine administration in Group 1 to the first participant in Group 2 to receive their first vaccine administration therefore allows continuous monitoring of the safety of both the second (D56) and third (D168) vaccinations in Group 1 participants receiving low dose IMP prior to the respective vaccinations in Groups 2.

The medically qualified investigator will be asked to provide the decision on whether to proceed to second (D56) and third (D168) vaccine administration in Group 2 after reviewing 7 days of blinded safety data after the last participant receives the second and third vaccine administrations in Group 1 respectively (See section 11.14). This review will consider blinded safety data from

the results of participant e-diaries, eCRFs, AEs after vaccination and safety bloods. This review will be based on the clinical judgement of the investigator with the option to escalate to the DSMC if any concerns. A similar review will be conducted using the safety data from the last participant to receive their second (D56) and third vaccine (D168) administrations of Group 2 prior to proceeding the the second (D56) and third (D168) vaccine administration of Group 3.

Cumulative toxicity has not been observed with similar GMMA based *Shigella* vaccines and is not expected with this vaccine. Thus staggered vaccination of cohorts of second and third vaccines in Group 1 and 2 will not be performed.

Full details about the reporting of any adverse events or serious adverse events, and the role of the DSMC beyond the dose escalation reviews is discussed in section 11 of the protocol.

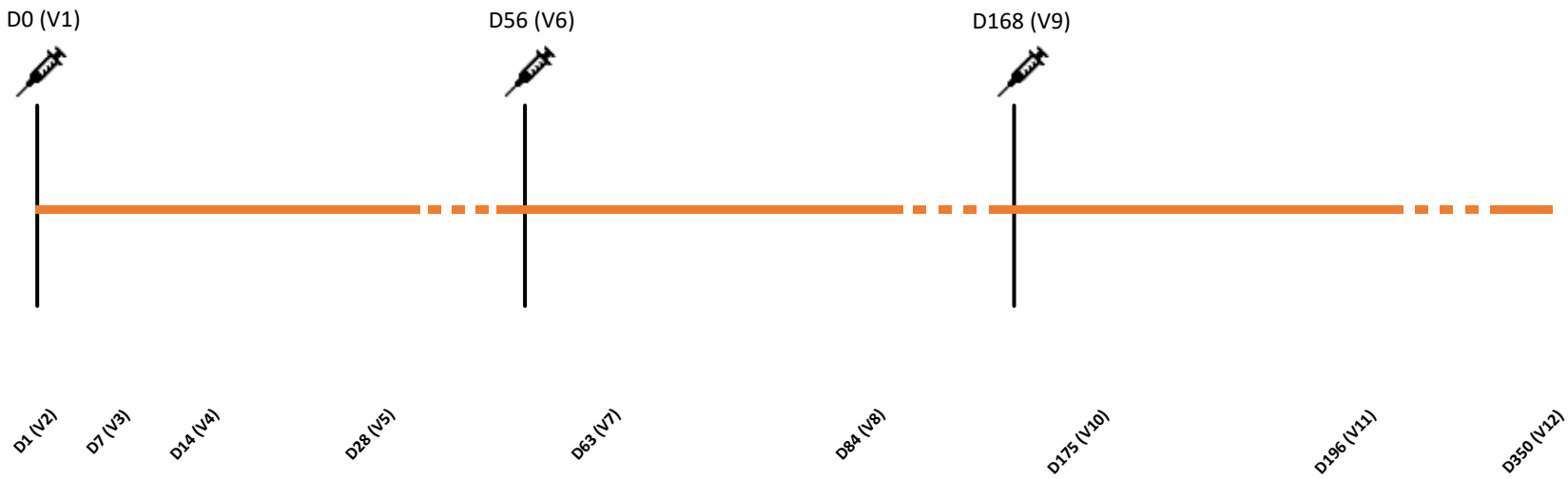
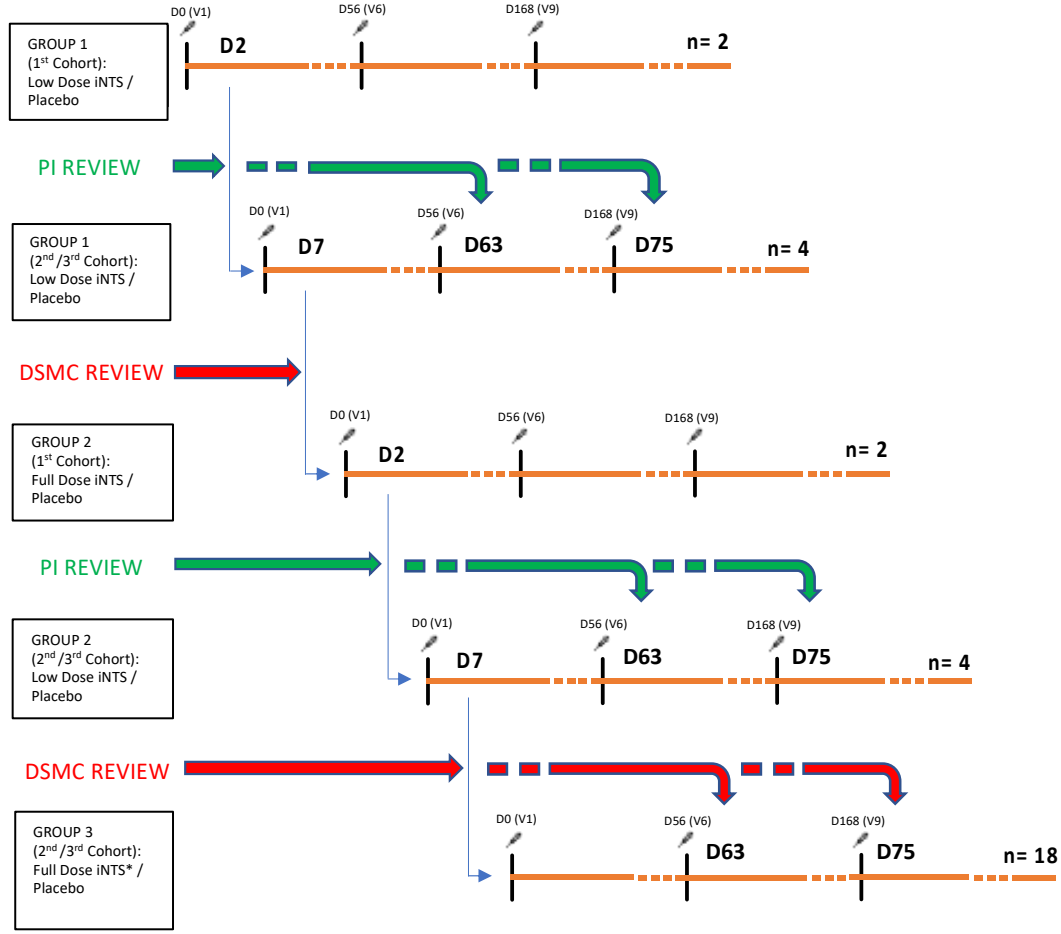


Figure 2.1 Vaccine schedule: Groups 1 – 3



* Low Dose iNTS may be considered if unacceptable safety signal in Group 2 high dose iNTS following Investigator and DSMC review

Figure 2.2 Dose Escalation Schedule: Groups 1 - 3

	Screening	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Indicative Study Day		D0	D1	D7	D14	D28	D56	D63	D84	D168	D175	D196	D350
Day post last vaccine		0	1	7	14	28	56	7	28	112	7	28	182
Visit Window (days)	V1 - 90*		0	+/- 1	+/- 2	+/- 7	+/- 4	+/- 1	+/- 7	+/- 4	+/- 1	+/- 7	+/- 28
Informed consent	x												
Confirmation of eligibilty criteria		x					x			x			
Obtain 24 hr contact details		x					x			x			
Medical history (including demographics and medication)	x												
Interim medical history (including concurrent medication)		x	x	x	x	x	x	x	x	x	x	x	x
Review / collection of AEs and SAEs since last visit		x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x												
Vital signs	x	x	x	x	x	x	x	x	x	x	x	x	x
Urine pregnancy test	x	x					x			x			
Urine dipstick (with urine analysis as needed)	x												
Blood sample	x	x	x	x	x	x	x	x	x	x	x	x	x
Oral Fluid Swab		x			x	x	x	x	x	x	x	x	x
Stool/Faecal Sample (Optional Only)		x										x	x
Vaccination		**x**					**x**			x			
e-Diary entries		x	x	x			x	x		x	x		
Intervention arm allocation		x											

Table 2.1 Visit Structure: Groups 1 - 3

Participants will complete the scheduled visits within the visit windows outlined above, however due to unforeseen circumstances such as participant unavailability, the visit may still proceed outside of window if reasonable to do so, as judged by the investigator. A physical exam is performed at screening. A physical exam is not routinely performed at the remaining visits unless the clinical history or situation deems it necessary.

*Once the screening visit has been completed, V1 must be scheduled within a maximum of 90 days of the date of the screening visit. This interval allows for receipt of GP medical summaries or repeat blood tests. If the V1 for the potential participant falls outside of this period, a repeat screening visit must be performed if the participant remains eligible and willing. However certain tasks which are unnecessary to be repeated as judged by the investigator may be brought forward. Tasks which must be repeated at this re-screening visit include: consent, interim medical history and urine pregnancy test (if applicable). Other tasks may also be performed at the Investigator's discretion.

8 PARTICIPANT IDENTIFICATION

8.1 TRIAL PARTICIPANTS

Male or female participants aged 18-55 years inclusive who are in good health (as determined by a study doctor) and who are able to provide written informed consent, will be eligible for inclusion in this study. Between 30-42 participants are required.

8.2 INCLUSION CRITERIA

Participants must satisfy all of the following criteria to be considered eligible for the study:

- Willing and able to give informed consent for participation in the study
- Aged between 18 and 55 years inclusive
- In good health as determined by
    - Medical history
    - Physical examination
    - Clinical judgment of the investigators
- (Females) Willing to use highly effective contraception as defined in Section 8.5 from one month prior to receiving the first vaccine and for the duration of the study
- Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
- Willing to allow his or her General Practitioner and/or Consultant, if appropriate, to be notified of participation in the study.
- Willing to allow study team access to medical records for the purposes of eligibility assessment and / or safety follow up during the trial.
- Willing to provide their national insurance number or passport number to be registered on The Over-Volunteering Prevention System (TOPS).

8.3 EXCLUSION CRITERIA

The participant may not enter the study if any of the following apply:

- History of significant organ/system disease that could interfere with the trial conduct or completion in the clinical judgement of the investigators. This includes any history of **significant** disease in the following:
    - Cardiovascular disease including congenital heart disease, previous myocardial infarction, valvular heart disease (or history of rheumatic fever), previous bacterial endocarditis, history of cardiac surgery (including pacemaker insertion), personal or family history of cardiomyopathy or sudden adult death
    - Respiratory disease such as uncontrolled asthma and chronic obstructive pulmonary disease
    - Endocrine disorders such as diabetes mellitus and Addison's disease
    - Significant renal or bladder disease
    - Biliary tract disease

    - Gastro-intestinal disease such as inflammatory bowel disease, abdominal surgery within the last two years, coeliac disease and liver disease (including hepatitis B or C infection)
    - Neurological disease such as seizures and myasthenia gravis
    - Haematological disease including coagulation problems
    - Metabolic disease such as glucose-6-phosphate dehydrogenase deficiency
    - Psychiatric illness requiring hospitalisation
    - Depression, anxiety or other psychiatric illness whose severity is deemed clinically significant by the study investigators
    - Known or suspected drug and/or alcohol misuse (alcohol misuse defined as an intake exceeding 42 units per week)
    - Non-benign cancer, except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ
- Have any known or suspected impairment or alteration of immune function, resulting from, for example:
    - Congenital or acquired immunodeficiency (including IgA deficiency)
    - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
    - Autoimmune disease
    - Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy (including for more than 7 days consecutively within the previous 3 months).
- Study significant abnormalities on screening investigations, that are either unlikely to resolve or do not resolve on repeat testing (at the discretion of an Investigator) within the recruitment timeline of the study
- Have received any oral typhoid vaccination (e.g. Ty21a or M01ZH09) within the last 3 years or a paratyphoid vaccine (as part of a clinical trial)
- Have participated in previous typhoid or paratyphoid challenge studies (with ingestion of challenge agent).
- Receipt of a live vaccine within 4 weeks prior to vaccination or a killed vaccine within 7 days prior to vaccination
- Plan to receive any vaccine other than the study vaccine within 4 weeks after any study vaccination (COVID-19 vaccine exempt, see Section 9.14)
- Any history of allergy or anaphylaxis to a previous vaccine or vaccine component
- Receipt of immunoglobulin or any blood product transfusion within 3 months of study start
- Participation in another research study involving an investigational product or that which may compromise the integrity of the study (e.g. significant volumes of blood already taken in previous study) in the past 12 weeks, or are planning to do so within the trial period
- Planned donation of blood/blood products outside of the study and during the trial period.
- Inability, in the opinion of the Investigator, to comply with all study requirements including likelihood of successful venepuncture during the trial
- Female participants who are pregnant, breastfeeding/lactating or planning pregnancy during the course of the study[1]

[1] As defined by CTFG Recommendations related to contraception and pregnancy testing in clinical trials, current document: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf [accessed 23rd March 2022]

- Weight less than 50kg or a BMI < 18.4 kg/m^2 or a BMI > 40 kg/m^2
- Any other significant disease or disorder which, in the opinion of the Investigator, may:
  - Put the participants at risk because of participation in the study
  - Influence the result of the study
  - Impair the participant's ability to participate in the study

8.4 TEMPORARY EXCLUSION CRITERIA

The following applies to both **initial enrolment** and **subsequent vaccination** visits. If the temporary exclusion resolves within the time constraints of the trial, they can be enrolled and/or progression in the trial can continue.

- Receipt of any systemic corticosteroid (or equivalent) treatment within 14 days prior to vaccination, or for more than 7 days consecutively within the previous 3 months
- Febrile illness (oral temperature ≥37.5°C) or systemically unwell on the day of vaccination
- If a participant is taking systemic antibiotics then the vaccination is postponed until 7 days after the last dose. This does not apply to topical antibiotic preparations
- Use of antipyretics in the 4 hours prior to vaccination
- A laboratory AE considered, in the opinion of the Investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered
- Symptoms of COVID-19, without confirmation of infection (as per current government guidelines) 14 days prior to vaccination visit
- Validated positive (first of episode) SARS-CoV-2 test (NAAT or antigen) within 4 weeks prior to vaccination visit
- Any illness / AE considered, in the opinion of the investigator, requiring of further time and/or investigation to resolve or stabilise prior to a dose of vaccine being administered

8.5 PREGNANCY AND CONTRACEPTION

The possible adverse effects of the iNTS-GMMA vaccine on the outcome of pregnancy are unknown; therefore, pregnant women will be excluded from the study. Women of childbearing potential will be required to use an effective contraceptive measure. Contraception should be maintained during the vaccination period and for the duration of the study. Should a volunteer become pregnant during the trial, she will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. We would not routinely perform venepuncture in a pregnant volunteer unless there is clinical need.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible. The active components of the iNTS-GMMA vaccine are the GMMA particles which consist of blebs of the outer membrane of the two Salmonella serovars, S.Enteritidis and S.Typhimurium. As a result, both components lack the necessary machinery for replication in vivo. They have been designed to stimulate an immune response to the antigen(s) contained within the GMMAs. Together with the lack of replicative machinery and a maximum iNTS-GMMA dose of 40µg makes the risk of human teratogenicity/fetotoxicity possible/unlikely.

A woman is considered of childbearing potential, i.e fertile, following menarche and until becoming post- menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophrectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhoea, a single FSH measurement is insufficient.

Female volunteers of childbearing potential are required to use a highly effective form of contraception until their last follow-up visit. Acceptable forms of contraception for female volunteers include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral/intravaginal/transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral/injectable/implantable)
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised male partner
- sexual abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

9 TRIAL PROCEDURES

9.1 RECRUITMENT

Identification of study participants

In order to recruit the required cohort of 30-42 participants, several strategies may be employed, including but not limited to:

- Poster advertising: Display of posters advertising the study throughout local hospitals and doctor's surgeries, tertiary education institutions and other public places with the permission of the owner/ proprietor.
- Direct mail-out / SMS/text message / emails: Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases. These include the National Health Applications and Infrastructure Services (NHAIS) via a NHAIS data extract or equivalent. For the NHS databases initial contact to potential participants will not be made by the study team. Instead study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd (or equivalent company), in order to preserve the confidentiality of potential participants. CFH Docmail Ltd (or equivalent company) is accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70). For mail-outs via the electoral register, we will have access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH

Docmail system (or equivalent company), and the study invitation pack will be sent out by CFH Docmail (or equivalent company). Volunteers may also be recruited using direct SMS/text message, or emails to potential participants identified by GPs from their databases.

- Email campaign: We will contact representatives of local tertiary education establishments and local employers and ask them to circulate posters and link to study website by email or hard copy.
- Oxford Vaccine Centre (OVC) database for healthy volunteers: Direct email and link to members of the public who have registered their interest in potentially volunteering for clinical trials conducted by OVC. This secure database is maintained by OVC and members of the public registered here have given consent to have their details recorded and be contacted expressly for this purpose of being notified when a trial opens for recruitment. They understand this is not a commitment to volunteering for any trial they are contacted about.
- Media advertising: Local media, newspaper and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- Website advertising: Description of the study and copy of information booklet on the OVG website.
- Social media: Advertisements placed on OVG or University of Oxford Social media accounts or targeted social media platform advertisements including, but not restricted to, Twitter, Facebook and Instagram
- Exhibitions: Advertising material and/or persons providing information relating to the study will exhibit using stalls or stands at exhibitions and/or fairs, such as University Fresher's Fairs.

Recruitment, approach and initial eligibility assessment of potential study participants
Potential participants who are interested in the study will be able to contact the OVG by telephone, email or trial website for further information. Once an expression of interest has been received by OVG, an information sheet will be sent via mail, email or downloaded from the website by potential participants to read at their leisure. If participants are willing to proceed, they will be initially screened by a website questionnaire and/or telephone before they are invited for a screening and consent visit, where their eligibility will be assessed by member of the clinical research team at the Oxford Vaccine Group. Permission to access the volunteer medical records either via the electronic records system or GP will be sought (if possible) prior to the screening visit. Participants will be asked to sign a secure electronic document (hosted by the REDCap database) which will then be counter-signed by a study team member. Alternatively written permission to access medical records will be sought at the volunteers screening and consent visit.

9.2 INFORMED CONSENT

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Consent will be sought as described in relevant SOPs.
Written and verbal versions of the participant information booklet and informed consent form will be presented to the participant, detailing no less than:

- the exact nature of and the rationale for performing the study
- implications and constraints of the protocol

- the risks and benefits involved in taking part

It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. The participant will be allowed at least 24 hours to consider the information from when they receive it, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study.

The participant will have the opportunity to discuss the study with a medically qualified investigator. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the appropriately trained and delegated clinician. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site.

Participants will be informed that they would also be eligible for BioBank ('Oxford Vaccine Centre Biobank' Southampton & South West Hampshire LREC (B) 10/H0504/25). BioBank is a separate study and optional to all participants of studies conducted by OVC. Separate consent is sought for this.

9.3 BASELINE ASSESSMENTS AT SCREENING

Once informed written consent is obtained, the following baseline assessments and information is collected as part of the assessment of inclusion/exclusion criteria:

- Participant demographics; age, sex and ethnicity
- Travel history (record travel to any country outside of the UK for longer than 2 months; record any travel to Sub-Saharan Africa or South East Asia)
- Medical history
- Contraception; female participants are asked if they are willing to use effective contraceptive measures one month prior to vaccination and for the remainder of the study
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- Recording of resting pulse, blood pressure, temperature, weight and height
- Physical examination; cardiovascular, respiratory, abdominal and gross neurological examination
- Urine dipstick (and laboratory analysis if appropriate) and urine pregnancy test
- Blood samples for: haemoglobin count, white cell indices, platelet count, serum sodium, serum potassium, serum urea, serum creatinine, liver function tests, C-reactive protein, HIV, Hepatitis B and C.
- Collect emergency contact details

The medical, vaccination and prescribed medication history are initially based on participant recall. However, with prior participant approval, patient medical summary, vaccination and prescribed medication history will be formally requested from the GP or accessed via the electronic patient record (if available) at the screening visit if not already requested or accessed in advance. In addition, all participant GPs will be notified of their participation in the study.

Consent will be taken to register the participant on The Over-volunteering Prevention System (TOPS) database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs and blood donations. This will be done using the

participant's National Insurance number or Passport Number. The TOPS database will be checked for any conflicts at screening, however formal registration will be done at enrolment.

9.4 RANDOMISATION

Groups 1 and 2 will be cohorted in pairs randomised 1:1 to receive the lower dose or full dose iNTS GMMA vaccine respectively versus placebo. Once the study has progressed to Group 3, 18 participants will be randomised 2:1 to receive the investigational product versus placebo.

Randomisation will be conducted using an electronic system within the RedCAP database. Participants in Group 1 and 2 will be randomised 1:1 in blocks of two, to meet the total numbers required for each group. Participants in Group 3 will be randomised 2:1 in blocks of three, to meet the total numbers required.

9.5 BLINDING AND CODE-BREAKING

This study will be conducted in an observer and participant blind fashion. The study blind will be maintained from the time of participant randomisation until participant unblinding which will occur once the last participant has completed their final visit.

There will be dedicated blinded and unblinded study teams. Blinded staff will include clinical study doctors (including the CI), study nurses, administrative and laboratory staff who will be directly managing participants and participant samples. Vaccine and placebo will be reconstituted, checked and administered by a dedicated unblinded study team, such that the participant will not be aware of which vaccine they have received. Assays requiring blinding in the laboratory (eg Elispots), will be measured by at least one individual blinded to vaccine or placebo allocation.

Participants and their General Practioners will receive written notification by letter or email of whether they have received the vaccine or placebo at the time of full study unblinding.

Unblinding may also occur at an earlier time point in the event of the occurrence of SAEs, SARs or SUSARs (please see section 11).

In the case of medical emergency the investigator will have direct access to unblinding of the participant(s) by opening of the participant(s) sealed envelope containing their vaccine record. This will be confirmed by electronic unblinding via the REDCap database, which can be performed in the first instance to avoid any delay in unblinding due to a medical emergency or out of hours.

This will be conducted under the guidance of the Data Safety and Monitoring Committee. Unblinding procedures will be conducted in accordance with local OVG SOPs.

9.6 VACCINATION VISITS

Vaccination visits are held at the CCVTM. The visit procedure for the vaccination visits will be as follows:

- Ensure that participant consent remains valid and confirm continued consent.

- Measure Weight, Height and Body Mass Index
- Obtain and document interim medical history since the last visit and check eligibility criteria, specifically temporary exclusion to vaccination
- Review for AEs and SAEs since the last visit
- Training on electronic diary card entry and if second or third vaccine review of diary card entries and laboratory AE profile
- Record oral temperature, pulse and blood pressure
- Perform urinary pregnancy test for females
- Perform blood draw
- Perform oral fluid swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Administer vaccine by IM injection into non-dominant deltoid muscle by the second team member in second clinic room (who remains unblinded, following iNTS-GMMA vaccine preparation as per clinical study plan)
- Observe for immediate adverse events for 60 minutes, followed by post vaccine checks including routine observations, review of vaccine site and assessment of wellbeing
- Schedule next visit and re-iterate participant requirements such as return of the Diary Card entries

9.7 NON-VACCINATION VISITS

Other visits may require the following procedures:

- Obtain and document interim medical history since screening and check continued eligibility
- Review for AEs and SAEs since the last visit
- Review eDiary entries and laboratory blood tests
- Record oral temperature, pulse and blood pressure
- Perform blood draw
- Perform oral swab
- Optional collection of stool sample +/- supply 'By Post' stool collection kit as required
- Schedule next visit and re-iterate participant requirements such as eDiary entries

9.8 OUTSIDE OF CCVTM VISITS

Participants will be asked to maintain a diary card describing all (solicited and unsolicited) adverse events up to seven days post vaccination. If there is an ongoing adverse event recorded in the diary the participant will be asked to continue with ediary entries until resolution depending on the nature of the adverse event and feasibility to do so as judged by the clinical investigator. Laboratory results are also entered into a safety results database in real-time or on demand for active monitoring throughout the study by a member of the study team. Each participant will be able to access a member of the study team 24-hours per day via a study-specific emergency number should they have any concerns or are in need of advice.

9.9 LABORATORY INVESTIGATIONS

In addition to blood samples needed for the safe conduct of the trial and assessment of the primary endpoint, blood/oral fluid/stool samples from the participants will also be subjected to laboratory analyses in order to assess the objectives defined in the secondary and exploratory endpoints. The plan for analysis is outlined below, and will be further detailed in a specific analysis plan:

9.9.1 ANALYSIS OF BACTERICIDAL ACTIVITY (GVGH)

The ability of the antibodies in participants serum samples to mediate killing of *S.* Typhimurium and *S.* Enteritidis in the presence of complement (serum bactericidal activity (SBA), will be quantified. The target strains in the SBA assay will be one wild-type strain per serotype in order to elucidate the antigen-specific SBA using a high-throughput luminescence assay developed by GVGH.

9.9.2 FURTHER ANALYSIS OF FUNCTIONAL ANTIBODIES (OVG and Collaborators)

The ability of the antibodies in participants' serum samples to mediate killing of a panel of *Salmonella* bacteria to assess cross protection of the iNTS-GMMA Vaccine. Further exploratory antibody analyses including but not limited to SBAs, opsonophagocytic assay and Fc glycosylation may be performed by OVG and collaborators.

9.9.3 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST O-ANTIGENS (GVGH)

Serum IgG antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. Test samples will be analysed at three dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Anti-OAg responses will be expressed in ELISA units. Plate coating antigens are well characterized OAg purified by GVGH.

9.9.4 ANALYSIS OF ANTIBODY CONCENTRATIONS AGAINST PORIN AND OTHER ANTIGENS (OVG and Collaborators)

Antibody responses against other antigens from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point may be analysed by ELISA developed and performed by OVG and collaborators. Test samples may be analysed at multiple dilutions and colour change compared with a standard curve made with calibrated human serum pool, included on each assay plate. Antigen responses will be expressed in ELISA Units.

9.9.5 CELLULAR RESPONSES AND CYTOKINE RELEASE (OVG and Collaborators)

Laboratory analyses to quantify the B-cell and T-cell responses specific to STmGMMA and SEnGMMA components of the vaccine will be performed when feasible using peripheral blood mononuclear cells (PBMCs) derived from study participants sampled before, and at several time points after each dose, using the assays described below.

9.9.6 ANALYSES OF B CELL RESPONSES (OVG and Collaborators)

The ability of the STmGMMA, SEnGMMA and the iNTS-GMMA vaccine to stimulate a detectable increase in antigen-specific memory B cells and plasma cells will be enumerated by ELISPOT using plates coated with vaccine antigens (such as individual GMMA or their

respective purified O antigen) or B cell mitogens. The phenotype and kinetics of the B-cell subsets involved in the response will be determined using fluorescent-labelled antibodies in a flow cytometric assay. In addition, other assays to monitor the B-cell immune response to the vaccines may be performed if sufficient samples are available.

9.9.7 ANALYSES OF T CELL RESPONSE AND CYTOKINE RELEASE (OVG and Collaborators)

In order to evaluate the ability of the iNTS-GMMA vaccine to stimulate T cell responses, we aim to quantify when possible vaccine-induced responding T-cells by multicolour flow cytometry and mass cytometry. Effector T cells will be clones and rested for antigen specificity against iNTS bacterial strains and tested for their capacity to recognise proteins contained within individual GMMAs. Moreover, other assays to monitor the T-cell immune response and cytokine release to the vaccines will be performed if sufficient samples are available.

9.9.8 ANALYSIS OF GENE EXPRESSION (OVG and Collaborators)

RNA will be extracted from a small volume of peripheral blood (~1ml) at three study visits (as per Table 3.1) for analysis of gene expression profiles. This analysis will be used to highlight differences in gene expression induced by vaccination and provide insight into the immunobiology of vaccine responses.

9.9.9 ANALYSIS OF GENETIC DETERMINANTS OF VACCINE RESPONSE (OVG and Collaborators)

DNA samples obtained, from peripheral blood, will contribute to a Biobank of samples from multiple different Oxford Vaccine Group studies. These DNA samples will be used to analyse the genetic factors influencing vaccine responses (immunogenicity and reactogenicity). DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses. This specific goal will therefore not contribute to the results of this individual study.

9.9.10 ANALYSIS OF ORAL FLUID ANTIBODY CONCENTRATION AGAINST O ANTIGENS AND PORINS (OVG and Collaborators)

Oral fluid samples will be collected via an oral fluid swab as detailed in the Clinical Study Plan. Antibody responses against OAg and porin Ag from *S.* Typhimurium and *S.* Enteritidis in samples from all subjects at each time point will be analysed by ELISA. OAg and Porin Ag responses will be expressed in Antibody Units.

9.9.11 COLLECTION OF SERUM TO BE USED AS A REFERENCE STANDARD FOR THE SET-UP OF LABORATORY ASSAYS IN CURRENT / FUTURE STUDIES (OVG/GVGH and Collaborators)

Serum samples (30mls) will be collected at Visit 8 (28 days post 2nd Vaccine) from recipients of the iNTS-GMMA vaccine within group 3. Serum will be processed as per OVG SOPs and stored at -80°C prior to transfer to GVGH for the development of the serum standard. Serum IgG responses to *S*. Typhimurium and *S*. Enteritidis will be screened by O Antigen ELISA. Samples

will be selected based on ELISA optical density (OD), characterised, calibrated and pooled. The calibrated human serum pool (or serum standard) will allow development of an ELISA standard curve by which test samples may be compared for quantification of antibody titres.

9.9.12 ANALYSIS OF FAECAL ANTIBODY CONCENTRATION AGAINST O ANTIGENS (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing a stool/faeces sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOP. Antibody responses against OAg from *S.* Typhimurium and *S.* Enteritidis will be analysed by ELISA. OAg responses will be expressed in Antibody Units.

9.9.13 INVESTIGATION OF IMPACT OF VACCINATION ON GUT MICROBIOTA (OVG and Collaborators)

This will be an optional study procedure for those participants who opt-in to providing said sample and has no bearing on ongoing participation in the study. Stool will be collected using specific containers following the established SOPs for bacterial whole genome sequencing and 16S RNA sequencing.

9.10 SAFETY BLOOD TESTS

All other laboratory tests including FBC, WBC differential counts, C-reactive protein, urea, creatinine, electrolytes, aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, will be performed using the OUHFT, NHS laboratories. Blood samples will be collected in assay sample tubes and delivered to OUH clinical laboratories for analysis according to national SOPs.

Samples collected as part of this study may also be used for other exploratory studies of scientific relevance by the OVG laboratory or any of the collaborating laboratories which may include the transfer of samples within and outside the EU. These samples may include oral fluid, serum, extracted DNA and RNA, and PBMCs. Frozen samples will be stored under the ethical approval for this study until study completion. At this time, samples will be transferred to the Oxford Vaccine Centre Biobank subject to participant consent (see Section 9.2). Studies may include further investigation of the inflammatory and immunological response to vaccination.

9.11 TRIAL PROCEDURE SCHEDULE

Details of which assays are performed on each visit are recorded in the table below. The total volume of blood obtained per patient over the course of the study will be 637.5 – 667.5mls.

Visits	Days	Vaccine / Post Vaccine Days	Antibody Assay: SBA, OAg (GSK)	Antibody / Porin Assays (OVG)	PBMCs (T Cell assays)	PBMCs (B Cell assays)	Functional Antibody Assays / Extracted from PBMCs	Serum Standard*	Transcriptomics	FBC	U&E, LFT, CRP	Viral Serology	Oral OAg/Porin IgG/A Assays	Stool/Faecal Ig Assays/Microbiota	Total (mls)
Screen	-	-	-	-	-	-	-	-	-	1	2	4	-	-	7
V1	D0	Vaccine 1	10	5	50	-	+	-	2.5	1	2	-	+	+	70.5
V2	D1	+1	-	-	-	20	-	-	2.5	-	-	-	-	-	22.5
V3	D7	+7	10	-	24	10	-	-	2.5	1	2	-	-	-	49.5
V4	D14	+14	-	5	24		-	-	-	-	-	-	+	-	29
V5	D28	+28	10	5	24	20	+	-	-	1	2	-	+	-	62
V6	D56	Vaccine 2	10	5	24	20	+	-	-	1	2	-	+	-	62
V7	D63	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V8	D84	+28	10	5	24	20	+	30*	-	1	2	-	+	-	62 (92*)
V9	D168	Vaccine 3	10	5	24	20	+	-	-	1	2	-	+	-	62
V10	D175	+7	10	5	24	10	-	-	-	1	2	-	+	-	52
V11	D196	+28	10	5	24	10	+	-	-	1	2	-	+	+	58
V12	D350	+182	10	5	24	10	+	-	-	-	-	-	+	+	49
															637.5 (667.5*)

Table 3.1: Blood Sampling (Including Oral Swab) Schedule (in mls)
***Serum standard (30mls) will be collected from participants in Group 3 only**
** Stool Sample collection will be participant opt-in only

Sampling time points, volumes and investigations may vary at the discretion of the PI. Samples may be omitted as per the investigating team's discretion for example if participants develop low haemoglobin as defined and managed as per the SALVO clinical study plan.

9.12 EARLY DISCONTINUATION / WITHDRAWAL OF PARTICIPANTS

Each participant can exercise their right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for participant safety including, though not exclusive to, the following:

- Significant non-compliance with study requirements
- Consent withdrawn
- Lost to follow up

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. The reason for withdrawal, if given, will be recorded in the CRF. A participant who is withdrawn from the study can be replaced if the individual has not received any vaccine. Therefore, lost to follow up subjects who have received at least one dose of vaccine will not be replaced. Furthermore, in circumstances pertaining to the safety of the participant, the

investigator may choose to discontinue further vaccination +/- study procedures, however with ongoing consent may continue to monitor for safety via either scheduled or unscheduled visits. Such circumstances may include though not exclusive to the following:

- Pregnancy
- An adverse event which requires discontinuation of the study vaccinations or results in an inability to continue to comply with study procedures
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. All data and participant samples obtained up to the point of withdrawal will be used in the analysis.

9.13 DEFINITION OF END OF TRIAL

The end-of-study is completion of the last laboratory assay on the last participant sample obtained at Visit 12. End of study must be achieved no later than 8 months after obtaining the last participant last sample at Visit 12.

9.14 SPECIAL CIRCUMSTANCES: COVID-19

9.14.1 STUDY CONDUCT / RISK ASSESSMENT

It is difficult to predict the time course of the COVID-19 pandemic. At all times the safety and welfare of study participants remains paramount.

The Chief Investigator will perform a risk assessment as necessary with relevant parties (e.g. DSMC, Regulatory Authorities or GVGH) on the basis of the current UK COVID-19 situation, to determine:

1. Appropriateness to initiate vaccinations
2. Appropriateness to continue the trial once started
3. Necessity to extend trial duration

Dependant on the prevailing COVID-19 situation, the conduct of the trial may be modified in line with national policy in effect at the time in the interests of participant safety. Such measures may include but are not limited to:

- Pausing further vaccinations
- Modifying study visits and procedures (as detailed in the SALVO Clinical Study Plan) eg: study visits conducted by phone or video calling where appropriate.

Any deviation not outlined in this protocol required due national policy in effect at the time, will require a non-substantial/substantial amendment unless specifically permitted to do otherwise by the Sponsor, MHRA and REC.

9.14.2 COVID-19 INFECTION CONTROL MEASURES AT VISITS

The CCVTM was one of the sentinel sites used by the University of Oxford to implement COVID-19 infection control policies and act as a model for securing a workplace. Further details of infection control procedures including the safe handling of clinic visits, a COVID-19 secure workplace, maintaining staff safety are included in the clinical study plan and OVG SOPs. These will be regularly updated in line with University of Oxford and NHS/UK Government policies.

9.14.3 PARTICIPANTS UNDER QUARANTINE

Given the evolving epidemiological situation both globally and in the UK, should a participant be under quarantine and unable to attend any of the scheduled visits, a telephone/video consultation may be arranged in order to obtain safety and the visit may be re-scheduled, depending on the timelines.

9.14.4 PARTICIPANTS WITH COVID-19 SYMPTOMS

Participants who become symptomatic during follow-up will be instructed to call the study team who will then advise on how to proceed with clinical testing for COVID-19, through the community testing programme, if necessary, as per the Clinical Study Plan. Participants would be expected to report a transient, flu-like illness within 24hours of vaccination. If this reaction should include a fever, we would expect this to resolve within 48hours. If a fever starts and resolves within 48hours of vaccination it will be attributed to the vaccine. If a fever persists for more than 48hours, or starts more than 48hours after vaccination, it will be considered unlikely to be related to vaccination and the participant may be advised to proceed with clinical testing for COVID-19 outside of the study if appropriate.

Participants who develop COVID-19 symptoms and have a positive SARS-CoV-2 test (appropriately validated NAAT or antigen test) after the first vaccination can only receive a subsequent vaccination after a minimum 4 weeks interval from their first positive test of that episode, provided they have had only a mild illness and have fully recovered. Moderate-Severe illness will be defined as 4 or above as per the WHO Clinical Progression Scale (See Appendix D: WHO Clinical Progression Scale for clinical studies of COVID-19). Those who have had moderate-severe disease will not receive further IMP.

In cases of mild or asymptomatic disease, the decision to proceed with booster vaccinations will be at clinical discretion of the investigators, and each case will be evaluated by a study doctor before proceeding (including physical examination and peripheral oxygen saturation recording [Sp02]). The trial clinician must assess that the participant has fully recovered from their illness. Participants must have no ongoing symptoms that could be attributable to their COVID-19 illness and feel that they have fully recovered and are well. For participants who are asymptomatic and have a positive SARS-CoV-2 test (and who remain asymptomatic), a minimum of 4 weeks from positivity will be required before further vaccinations are administered. They will also undergo a physical examination and peripheral oxygen saturation recording before proceeding with IMP.

All relevant details and any required modification in study visits/procedures as per protocol will be documented in a separate adverse event eCRF. Management of these participants will be as detailed above and in the SALVO Clinical Study Plan. Participant follow up and safety reporting will be as detailed in Section 11 Safety Reporting.

9.14.5 PARTICIPANTS INVITED FOR COVID-19 VACCINATION DURING THE TRIAL

Participants who are yet to receive a COVID-19 vaccine but become eligible for this as per UK policy, would be invited to discuss this with the study team.. If agreed by the participant and if it were possible, we would find a mutually agreeable time to receive a COVID-19 vaccine in concert with study timelines and as per exclusion / inclusion criteria (section 8.3). Participants would not be impeded in taking up an offer of a COVID-19 vaccine if offered through the national rollout. If a rollout offer coincides with a planned trial vaccine, the trial vaccine would be rescheduled to 2 weeks before or after the COVID-19 vaccine (whichever was closest to trial schedules). This is in line with the UK Green Book COVID-19 vaccination recommendations for other vaccines which recommends "at least 7 days" interval. It would also minimise the risk of any cross-attribution of reactogenicity, and minimise impact on immunology investigations. If a participant receives a COVID-19 vaccine during the trial, both the vaccine and date of administration will be requested and recorded in the eCRF.

10 TRIAL INTERVENTIONS

10.1 INVESTIGATIONAL MEDICINAL PRODUCT (IMP) DESCRIPTION

10.1.1 iNTS GMMA VACCINE

The vaccine product iNTS-GMMA consists of 2 components: 80 µg OAg/mL STmGMMA and 80 µg OAg/mL SEnGMMA each asceptically formulated independently on Alhydrogel (0.7 mg AL^{3+} / mL) in isotonic 20mM Phosphate buffered saline pH 6.5. Prior to administration, equal volumes of each component are mixed as specified in the clinical study plan to yield:

- Full dose: 20 µg OAg of STmGMMA/Alhydrogel + 20 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL.
- Lower dose: 5.3 µg OAg of STmGMMA/Alhydrogel + 5.3 µg OAg of SEnGMMA/Alhydrogel in 0.5 mL (3.8 dilution of iNTS-GMMA vaccine full dose diluted with placebo).

10.1.2 PLACEBO

The placebo consists of 0.5 mL Alhydrogel in isotonic 20mM Phosphate buffered saline pH6.5. The placebo is also used for vaccine dilution.

10.2 BLINDING OF IMPS

Group 1-3 will operate a participant-observer blind i.e. both the participant and the observer will be unaware of the group allocation. There will be a trained unblinded team who will prepare and administer the iNTS and placebo vaccine. Both the iNTS and placebo vaccine will appear similar at the point of administration to the participant.

10.3 STORAGE OF IMP

The vaccine product requires storage at 2 to 8oC throughout and vaccines will be transported to the OVG after authorised release for use in the clinical trial by the GSK Qualified Person (QP)

and study approval by EC and MHRA. All movements of study medication between GVGH and OVG, will be documented in accordance with relevant SOPs.

The study treatment will be stored at the OVG in temperature monitored refrigerators with an auditable temperature record in accordance with the manufacturer's instructions and relevant SOPs. Study fridges are connected to a monitoring system with 24-hour access to staff that are able to move the product in the event of significant temperature deviation, for example fridge malfunction as per OVG 001: Vaccine Receipt, Storage, Cold Chain Maintenance and Return/Deposal.

10.4 COMPLIANCE WITH TRIAL TREATMENT

The study investigational product and placebo will be administered by trained unblinded study personnel and will be documented according to GCP guidelines and relevant SOPs. Issues related to compliance are therefore the responsibility of study personnel who have received appropriate training.

Access to the randomisation and vaccination eCRF of the study database will be password protected and restricted to the unblinded study team . In the unlikely event that accidental unblinding (i.e for reasons not outlined in this protocol) of any blinded study team member occurs, this would be recorded as a protocol deviation, and the study team member will be quarantined from taking part in further blinded study activities, in so far as the study progress is not compromised.

10.5 ACCOUNTBILITY OF THE TRIAL TREATMENT

iNTS-GMMA vaccines, placebo (also used as diluent) and empty vials will be manufactured, packaged, labelled and supplied by GVGH. All vaccines (vials and boxes) are labelled with a label specifying 'for clinical trial use only' and no less than the following:

- The clinical trial identifier (by reference code)
- The content of each vial
- Batch and serial number
- Chief Investigator
- Research site

The vaccine will be delivered and stored at the CCVTM pending authorised release for use in the clinical trial.

10.6 CONCOMITANT MEDICATION

The use of all concomitant medication prescribed or over-the-counter, will be recorded in the CRF. There is no restriction on the use of concomitant medication but the use of some prescribed medicines, such as immune suppressive agents, may result in the withdrawal of the participant at the discretion of the Investigator, while others, such as antibiotics, may result in a temporary exclusion.

10.7 EMERGENCY MEDICATION AND PROCEDURES

Participants are required to wait and be observed for one hour after the administration of each vaccine dose for signs of anaphylaxis. All clinical staff are trained and can provide evidence of competency in the acute management of anaphylaxis reactions including the use of intra-muscular adrenaline. This is detailed in relevant SOPs and adrenaline is available at all times of vaccine administration and subsequent observation.

The nearest Accident and Emergency Department is at the Oxford University Hospitals NHS Foundation Trust, which is within minutes by ambulance transfer.

10.8 POST-TRIAL TREATMENT

Study medication will not be continued beyond the trial period.

10.9 OTHER TREATMENTS (NON-IMPS)

No other treatments other than those specified in the protocol above will be administered to trial participants.

10.10 OTHER INTERVENTIONS

No other interventions other than those specified in the protocol above will be administered to trial participants.

11 SAFETY REPORTING

11.1 SAFETY REPORTING DEFINITIONS

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

11.2 CAUSALITY ASSESSMENT

The relationship of each adverse event to the trial vaccine(s) or study procedures must be determined by a CI-delegated blinded clinician / investigator. The relationship of the adverse event with the study procedures will be categorized as not related, possibly related, probably related or definitely related. The delegated clinician will use clinical judgement to determine the relationship using the following definitions:

Not related	• No temporal relationship to vaccine administration ***and*** • Alternative aetiology (clinical, environmental or other intervention), ***and*** • Does not follow pattern of recognised response to vaccine administration	
Related	Possible	• Reasonable temporal relationship to vaccine administration, ***or*** • Event not readily explained by alternative aetiology (clinical, environmental or other interventions), ***or***

		• Similar pattern of response to that seen to vaccine administration.
	Probable	• Reasonable temporal relationship to vaccine administration, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***or*** • Known pattern of response with vaccine administration.
	Definite	• Reasonable temporal relationship to vaccine administration or other study procedure, ***and*** • Event not readily produced by alternative aetiology (clinical, environment, or other interventions), ***and*** • Known pattern of response to vaccine administration.

11.3 SEVERITY ASSESSMENT

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on the criteria listed in the definition of an SAE in section 11.1 above. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Severity will be assessed by clinical symptoms, signs, diagnosis, laboratory results and observations as per the appendices A, B and C. Use of the appendices will be detailed in the following sections.

11.4 PROCEDURES FOR COLLECTING AND RECORDING ADVERSE EVENTS

Abnormal clinical findings from medical history, examination or blood tests, will be assessed by a blinded delegated clinician / investigator as to their clinical significance using the severity grading criteria for Adverse Events tables (see Appendix A, B, C).

All AEs that are observed by the investigator or reported by the participant irrespective of their relatedness to the study medication will be recorded from the day of vaccination and until 28 days after each vaccination These will be recorded in either the e-diary for the first 7 days after each vaccine, the laboratory safety database or the eCRF. Outside of this window (i.e. from 28 days after each vaccination and until the point of a subsequent vaccination or until the final visit if vaccination course completed), non-serious AEs will only be recorded if they require medical attention (contact with GP, visit to emergency department). These will be recorded in the eCRF. All AEs will be collected and recorded by the blinded study team.

It will be left to the blinded investigator clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from further vaccination / study. Such

judgement may require the unblinding of the investigator +/- the participant as per study procedures.

A participant may also voluntarily withdraw from the study due to what he or she perceives as an intolerable AE. In such an event, Section 9.10 (Early Discontinuation/Withdrawal of Participants) will apply. All AEs that result in a participant's withdrawal from the study will be, subject to participant consent, followed up where possible until a satisfactory resolution occurs, or until a non-study related causality is assigned. This will involve an end of study assessment at which the requirement for further appropriate care under medical supervision will be determined. If required the participant will be referred to their GP for ongoing medical supervision, until symptoms cease or the condition is deemed resolved or stable.

11.4.1 E-DIARY AEs

Solicited adverse events

Solicited AEs are those listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- Solicited adverse events will be recorded by the participant in an electronic diary and graded by the participant alone (appendix A) from the time of each vaccine administration for 7 days post-vaccination (day of vaccination and six subsequent days).

Solicited adverse events will be reviewed daily by the clinical study team. These are participant-entered. If further action is required including face-to-face medical review, and/or prescribed medication this will be recorded by the study team in the eCRF. Causality will be assigned by blinded CI-delegated clinician / investigator. Any solicited AE which meets the definition of a SAE will be managed and reported as per Section 11.6.

Unsolicited adverse events

Unsolicted AEs are those that are NOT listed as foreseeable adverse reactions to either iNTS-GMMA vaccine or placebo in section 11.9 below.

- These may be recorded by the participant in an electronic diary from the time of each vaccine administration for 7 days post vaccination.

Unsolicited adverse events will be reviewed at clinic visits. If clarification of any event is required then the study nurse or doctor will seek this from the participant during a clinical visit or by telephone call. These unsolicited adverse events will be recorded in the AE section of the eCRF. Unsolicited adverse events recorded in the e-diary will be severity graded by the participant. Causality will also be assigned by the CI-delegated clinician / investigator as per section 11.2.

Additionally participants will be asked about the occurrence of AEs during visits and if any are elicited (within the period for which the eDiary is open) that have not already been recorded they will be recorded in the eDiary as above.

11.4.2 OBSERVATION RELATED AEs

Physical observations (e.g. temperature, blood pressure) of the patient will be taken at each visit (Section 9.6, 9.7). These will be recorded in the eCRF. If abnormal, a severity grading will be automatically assigned by the blinded study team as per Appendix B.

11.4.3 VISIT ELICITED AEs

Participants will be asked about the occurrence of AEs and if any elicited they will be recorded in the eCRF and graded as per Appendix A and B by a blinded CI-delegated clinician / investigator. Any AEs reported outside of the visits will be recorded in the eCRF, except for AEs elicited during the opening period of the eDiary (see Section 11.4.1).

11.4.4 LABORATORY AEs

During the trial, laboratory results will be entered into a password protected database containing all the trial safety blood results. Severity grading for laboratory AEs is defined in Appendix C. Changes in laboratory values will be recorded as AEs if they are of Grade 2 severity or above. Changes of laboratory values of Grade 1 severity may be recorded as AEs if they are judged to be clinically significant by a blinded CI-delegated clinician / investigator.

If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and advised with regards appropriate medical care. Laboratory results may be out of normal range for a number of reasons (eg hot weather, delayed transit to processing laboratory). Changes in laboratory values will be recorded as AEs if they fall out of prespecified ranges and judged to be clinically significant by a blinded CI-delegated clinician / investigator, if some action (eg repeat testing for likely clinically significant test result, or reduction in blood volume required during blood draws) or intervention is required. There may be certain circumstances where it may be necessary to unblind the participant. Should this be necessary the same procedures will be followed as for unblinding participants for SAEs.
If abnormal laboratory values are the result of pathology for which there is an overall diagnosis then this diagnosis should be reported as one AE only.
A Grade 4 laboratory AE will be considered a SAE.

11.4.5 NOTES ON RECORDING AEs

Pre-existing medical conditions (present prior to enrolment into the study) are considered “concurrent medical conditions” and should not be recorded as AEs. However, if the participant experiences a worsening or complication of the condition, the worsening or complication should be recorded as an AE. Study staff will ensure that the AE term recorded captures the change in the condition (e.g., “worsening of”).

Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect at which point it would fall within the definition of "serious" and the congenital abnormality of birth defect would be reported as an SAE. Pregnancy notification and follow-up reports on pregnancy outcome will be provided to the DSMC with ongoing consent of the participant.

11.4.6 FOLLOWING UP OF AEs

AEs considered related to the active vaccine or placebo will be followed until resolution, the event is considered stable or until non-study causality is assigned. At the end of the study all other ongoing/open AEs will be assessed by a blinded CI-delegated clinician / investigator, to ensure if not already done so, adequate medical follow-up (if required) has been arranged, eg. referral to participant's general practitioner.

11.5 REPORTING PROCEDURES FOR SERIOUS ADVERSE EVENTS

SAEs will be collected throughout the entire trial period (from first vaccination to D350 or withdrawal).

All SAEs must be recorded on a SAE form (paper or electronic) with causality assessed by the blinded investigator and reported by email to the CI. All SAE will be reported to the DSMC Chair (or nominated designee) within 24 hours of discovery or notification of the event. If the SAE is deemed related, the CI will unblind to confirm whether the participant has received study vaccine or placebo. If the CI deems that this is a SUSAR, this will be reported according to the SUSAR reporting procedures below. In the absence of the CI these tasks may be performed by a Co-Investigator.

Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form emailed to the CI and DSMC Chair (or nominated designee).

The chair of the DSMC (or nominated designee) will perform an independent review of SAEs and request any further information required in a manner adherent to the procedures and timelines of the DSMC Charter. Documentation of this review will be kept in the TMF. The DSMC will provide independent real-time safety assessment throughout the study as described below.

11.6 EXPECTEDNESS

Expectedness will be determined according to the reference safety information section of the Investigators' Brochure for iNTS-GMMA vaccine. No IMP related SAEs are expected in this study. All SAEs at least possibly related to iNTS-GMMA vaccine will be considered unexpected and be reported to the MHRA and REC as SUSARs within the regulatory timelines, as in section 11.8.

11.7 SUSAR REPORTING

All SUSARs will be reported to the Sponsor, relevant Research Ethics Committee, GVGH, and to the MHRA. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. Any additional relevant information should be sent within eight days of the report.

The CI or Co-Investigator will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

11.8 FORSEEABLE ADVERSE REACTIONS

The foreseeable ARs following vaccination with iNTS-GMMA vaccine or placebo include, locally; injection site pain/ tenderness, redness, swelling, induration; and systemically, headache, malaise, fever, nausea, vomiting, abdominal pain, anorexia, myalgia, arthralgia, and fatigue.

11.9 DEVELOPMENT SAFETY UPDATE REPORTS

In addition to the expedited reporting above, the CI or CI-delegated study team member shall submit once a year throughout the clinical trial or on request, a Development Safety Update Report (DSUR) to the;

- MHRA
- Research Ethics Committee
- Sponsor (RGEA)
- GVGH

11.10 SAFETY PROFILE REVIEW

The safety profile will be reviewed on a day to day basis by the investigators using a blinded electronic diary, adverse events CRF and safety bloods to date. Any concerns will be referred to the blinded CI. If the CI remains concerned they may consider unblinding and/or escalation to the unblinded DSMC as required.

11.11 TRIAL MANAGEMENT GROUP

The OVG study investigators will form the trial management group (TMG) and will provide on-going management of the trial.

11.12 DATA SAFETY MONITORING COMMITTEE (DSMC)

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. Specifically, data review will be done as follows:

1. Formal review of the safety profile after 7 days of safety data has been collected from group 1 before progression to group 2, and review of group 2 after 7 days of safety data has been collected before progression to group 3, as described in section 7.
2. Formal review of the safety profile after two further timepoints unrelated to dose escalation decisions: at least seven days after the last participant in group 3 to receive the

second vaccine; and at least seven days after the last participant in group 3 to receive the third vaccine.

3. Independent review following any SAE deemed to be related to the trial active vaccine or placebo.
4. Unscheduled reviews on request of the study management committee at a demand and frequency determined by the severity of reported adverse events.

From these reviews the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. A summary of all blinded and unblinded AEs and SAEs to date will be provided to the DSMC on request.

The outcome of each DSMC review will be communicated directly to the TMG and documentation of all reviews will be kept in the TMF. The CI will inform GVGH of the outcome of the DSMC review.

The Chair of the DSMC will also be contacted for advice where the Chief Investigator feels independent advice or review is required.

11.13 OTHER SAFETY REVIEWS

In addition to formal DSMC review, there will be local blinded safety monitoring reviews. As described in section 7.2 and 7.5, there will be formal local blinded reviews of safety data by the CI or CI-delegated clinician / investigator at:

1. Day 2 following vaccination of the first paired cohort in groups 1 and 2, to decide on progression to vaccination of respective second and third paired cohort.
2. Following 7 days of safety data after the last participant to receive their second vaccine in group 1, to decide on progression to administer second vaccines in group 2.
3. Following 7 days of safety data after the last participant to receive their second vaccine in group 2, to decide on progression to administer second vaccines in group 3.
4. Following 7 days of safety data after the last participant to receive their third vaccine in group 1, to decide on progression to administer third vaccines in group 2.
5. Following 7 days of safety data after the last participant to receive their third vaccine in group 2, to decide on progression to administer third vaccine in group 3.

11.14 GROUP HOLDING RULES

Group holding rules are as follows:

SAE

- All Grade 3 adverse events in any individual which is possibly, probably or definitely related to vaccination (i.e. a AR) will be assessed by the CI-designated clinician / investigator to determine whether the event meets the criteria for a SAE related to vaccination (i.e. a SAR) as per Section 11.1. If this criteria is met the this would trigger the following group holding rule.
- An SAE which occurs in any one individual which is possibly, probably or definitely related to vaccination (i.e. a SAR) would trigger a group holding rule.

Solicited/unsolicited/laboratory adverse events

Solicited local adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 solicited local adverse event within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs

Solicited systemic adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any groups are followed by a Grade 3 solicited systemic adverse event beginning within 7 days after vaccination (day of vaccination and six subsequent days) and persisting at Grade 3 for >48 hrs.

Unsolicited adverse events:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 unsolicited adverse event within 7 days after vaccination (day of vaccination and six subsequent day) and persisting at Grade 3 for >48 hrs.

Laboratory adverse event:

- If 2 or more doses of the vaccine or placebo at a given time point (Day 0, Day 56, Day 168) within any group are followed by a Grade 3 laboratory adverse event beginning within 3 days after vaccination and not significantly improving (on clinical judgement), persistent or worsening on repeat testing at a clinically appropriate interval.

If the holding rule has been met and following a safety review by the DSMC it is deemed appropriate to restart dosing or to continue only with the lower dose of vaccine, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The DSMC safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Study Information Booklet (SIB) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The local ethics committee, MHRA, GVGH and the Sponsor will be notified if a holding rule is activated or released.

All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

11.15 INDIVIDUAL HOLDING RULES

In addition to the above stated group holding rule, stopping rules for individual volunteers will apply (i.e. indications to withdraw individuals from further vaccinations):

- **Local reactions:**
  - Injection site ulceration, abscess or necrosis

- **Laboratory AEs:**
  - the volunteer develops a Grade 3 laboratory adverse event considered related within 7 days after vaccination, not significantly improving (on clinical judgement), persistent or worsening on repeat testing.

- **Solicited adverse events:**
  - the volunteer develops a Grade 3 systemic solicited adverse event considered related within 7 days after vaccination (day of vaccination and six subsequent days), persisting continuously at Grade 3 for > 48hrs.

- **Unsolicited adverse events:**
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, persisting continuously at Grade 3 for > 48hrs,
  - the volunteer has a Grade 3 adverse event, considered related to vaccination, which is considered a serious adverse event as assessed by the CI-designated clinician / investigator,
  - the volunteer has a serious adverse event considered related to vaccination, or
  - the volunteer has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.

If a volunteer fulfils any of the temporary exclusion criteria (see section 7) at the scheduled time of a second administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol (see Table 1) or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated volunteers will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

11.16 STOPPING RULES

The trial will be discontinued in the event of any of the following:

- New scientific information is published to indicate that subjects in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the subjects enrolled in this or any other on-going trial of the GMMA vaccine delivery system.
- For any other reason at the discretion of the Chief Investigator or DSMC.

Additionally, the DSMC can temporarily pause the trial if time is required to reach a decision regarding stopping the trial e.g. to determine causality for SAE.

12 STATISTICS

12.1 DESCRIPTIVE STATISTICAL METHODS

The analyses for this study will be descriptive in purpose and will not include any hypothesis testing or presentation of p values for group comparisons or power calculation.

12.2 THE NUMBER OF PARTICIPANTS

30-42 participants will be recruited to the study allocated to groups 1-3 as detailed in section 7. Participants will be replaced only if they have not received a dose of vaccine. There has been no formal power calculation to determine this figure as the study is primarily descriptive. The number of participants has therefore been chosen to pragmatically reflect logistical and budgetary constraints.

12.3 THE LEVEL OF STATISTICAL SIGNIFICANCE

There will be no statistical significance testing. All confidence intervals for descriptive analyses will be set at 95%.

12.4 CRITERIA FOR TERMINATION OF TRIAL

The Chief Investigator and Data Safety Monitoring Committee will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the investigator will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the MHRA, GVGH and relevant Ethics Committee will be notified within 15 days of this occurring.

In the event of the trial being terminated early, follow-up of enrolled participants will still continue as detailed in tables 2.1 for safety reasons, with the exception that further vaccination will not be given and study procedures will be modified to monitor safety only.

12.5 PROCEDURE FOR ACCOUNTING FOR MISSING, UNUSED, AND SPURIOUS

All available data will be used in the analyses and there will be no imputations for missing data. Participants will be analysed according to the group to which they were assigned.

12.6 INCLUSION IN ANALYSIS

All participants with any available data will be included in the analyses.

12.7 INTERIM ANALYSIS

An interim analysis of the secondary objective, immunogenicity at two dose levels (lower dose and full dose iNTS-GMMA Vaccine) will be performed once enrolement is complete and once all second vaccine + Day 28 (Day 84; V8) samples have been collected from all groups. An interim analysis of some of the exploratory objectives may also be performed dependant on validation of the exploratory assays.

13 DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

The investigators will populate the content of participants' CRFs, which will be in a paper and/or electronic format using an REDCap database (or an appropriate alternative). This database is stored on a secure University of Oxford server and has restricted access and is password-protected with accountability records. This data includes safety data, laboratory data (both clinical and immunological) and outcome data. All information transcribed to and from the REDCap database is by encrypted (Https) transfer.

Each study participant will have a unique screening number which will be allocated following the taking of informed consent and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number which will be determined by enrolment order and initials as a second identifier. Samples sent to laboratories for processing will be identified by a trial number and participant number only.

13.1 DATA INTEGRITY

Data collection and storage will be inspected throughout the study by internal (performed by the Oxford Vaccine Group) and external (Appledown Clinical Research Ltd) monitoring. The Sponsor may also audit the trial data.

13.2 DATA ARCHIVING AND STORAGE

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked filing cabinet at the site. All essential documents will be retained for a minimum of 5 years after the study has finished. Volunteers who complete online screening or telephone screening only (before informed consent) will not have data kept beyond the end of the trial. The need to store study data for longer in relation to licensing of the vaccine will be subject to ongoing review. For effective vaccines that may be licensed, we may store research data securely at the site at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for 7 years in line with the site financial policy. De-identified research data maybe be stored indefinitely.

13.3 SOURCE DATA

Source documents are original documents, data, and records from which participants' CRF data is populated. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study CRF entries will be considered source data where it is the site of the original recording. All documents will be stored safely under strict confidentiality and with restricted access. On all study-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the study participant number/code only.

13.4 ACCESS TO DATA

Direct access will be granted to authorised representatives from the sponsor/host institution (including Appledown clinical Research Ltd), GSK/GVGH and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.5 DATA RECORDING AND RECORD KEEPING

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. REDCap, or similar) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment. REDCap is widely-used, powerful, reliable, well-supported system. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored in a separate password protected Access databased saved on a secure University of Oxford server. Only Oxford staff have access to the Access database and are permitted for data entry.

Each study participant will have a unique participant number which will be allocated at the time a screening visit is booked and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number and/or code. Samples sent to laboratories for processing will be identified by trial number and participant number only.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g. requesting to be informed of other trials), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Bank details will be stored for 7 years in line with University financial policy.

14 QUALITY ASSURANCE PROCEDURES

14.1 RISK ASSESSMENT

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities. Approved and relevant SOPs will be used at all clinical and laboratory sites.

14.2 MONITORING

Regular monitoring will be performed by Appledown Clinical Research Ltd according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

15 PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file as per SOP.

16 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.
A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority, the relevant NHS host organisation and GSK/GVGH within seven calendar days.

17 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 DECLARATION OF HELSINKI

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

17.2 GUIDELINES FOR GOOD CLINICAL PRACTICE

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

17.3 APPROVALS

Following sponsor approval the protocol, informed consent form, participant information sheet and required material will be submitted to an appropriate Research Ethics Committee (REC), MHRA, regulatory authorities, and host institution(s) for written approval.
The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4 TRANSPARENCY IN RESEARCH

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17.5 REPORTING

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and summary report will be submitted to the MHRA, the REC, host organisation and Sponsor.

17.6 PARTICIPANT CONFIDENTIALITY

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number and initials on all trial documents and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

17.7 PARTICIPANT REIMBURSEMENT

Each participant is compensated for their time and for the inconvenience based on the following figures:

- Travel expenses: £15 per visit
- Inconvenience of blood tests: £10 per visit
- Time required for visits: £20 per visit

Remuneration is on a *pro rata* basis should a participant fail to complete all visits and/or study requirements. Each participant can therefore receive a maximum of £585. Payments will be made in instalments after V0, V6, V10, and V12.
Additional reimbursement for unscheduled visits at £45 per visit will be provided. This will not be given unless an unscheduled visit occurs.

18 FINANCE AND INSURANCE

18.1 FUNDING

This clinical trial is funded by a European Union Horizon2020 grant. Additional budget from GVGH will cover necessary costs for monitoring activities not already funded by the Vacc-iNTS European Union Horizon2020 grant.

18.2 INSURANCE

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London

18.3 CONTRACTUAL ARRANGEMENTS

Appropriate contractual arrangements will be put in place with all third parties.

19 PUBLICATION POLICY

The Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator prior to submission.

20 DEVELOPMENT OF A NEW PRODUCT/ PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP derived from this trial will be in accordance with the Consortium Agreement signed by Beneficiaries of the Horizon2020 Vacc-iNTS EU Grant No 815437.

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APPENDIX A: GRADING THE SEVERITY OF SOLICITED AND UNSOLICITED ADVERSE EVENTS

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6
	1	37.6 – 38.0
	2	38.1 – 39.0
	3	> 39

Adverse event	Grade	Definition
Any symptom	0	Absence or resolution of symptom
	1	Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3	Significant interference with daily activity
	4	Emergency department visit or hospitalisation
	5*	Fatality

*All grade 5 AE will be considered either a SAE, SAR, or SUSAR dependant on causality and 'expectedness'

APPENDIX B: GRADING THE SEVERITY OF VISIT OBSERVED ADVERSE EVENTS

Observation	Grade 1	Grade 2	Grade 3
Oral temperature (ºC)	37.6 – 38.0	38.1 – 39.0	>39
Tachycardia (beats/min)	101-115	116-130	>130
Bradycardia (beats/min)	50-54	45-49	<45
Systolic hyper-tension (mmHg)	141-150	151-155	>155
Diastolic hyper-tension (mmHg)	91-95	96-100	>100
Systolic hypo-tension (mmHg)	85-89	80-84	<80

The following ranges are considered normal physiological ranges and are recorded as Grade 0:

- Oral temperature between 35.5 and 37.5 C
- Resting heart rate between 55 and 100 beats/minute
- Systolic blood pressure between 90 and 140 mmHg

APPENDIX C: GRADING THE SEVERITY OF LABORATORY OBSERVED ADVERSE EVENTS

Parameter	Grade 1	Grade 2	Grade 3	Grade 4*
Haemoglobin: decrease from baseline value (g/l)	≤ 15	16-20	21-50	>50
White cell count: elevated (10^9/L)	11.5–15	>15–20	>20–25	>25
White cell count: depressed (10^9/L)	2.5-3.5	1.5-2.49	1.0-1.49	<1.0
Neutrophil count (10^9/L)	1.5-1.99	1.0-1.49	0.5-0.99	<0.50
Platelets (10^9/L)	125-140	100-124	25-99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150
Potassium: hyperkalaemia (mmol/L)	5.1–5.2	5.3–5.4	5.5–5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.3–3.4	3.1–3.2	3.0	<3.0
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	114-156	157-312	>312	RRT
ALT and/or AST (IU/L)	1.1–2.5 x ULN	>2.5–5.0 x ULN	>5.0-10 x ULN	>10 x ULN
Bilirubin, with increase in LFTs (µmol/L)	1.1–1.25 x ULN	>1.25–1.5 x ULN	>1.5–1.75 x ULN	>1.75 x ULN
Bilirubin, with normal LFTs (µmol/L)	1.1–1.5 x ULN	>1.5–2.0 x ULN	>2.0–3.0 x ULN	>3.0 x ULN
Alkaline phosphatase (IU/L)	1.1–2.0 x ULN	>2.0–3.0 x ULN	>3.0–10 x ULN	>10 x ULN
Albumin: hypoalbuminaemia (g/L)	28–31	25–27	<25	Not applicable
C-reactive protein	>10-30	31-100	101-200	>200

Grade 4* Potentially life threatening

APPENDIX D: WHO CLINICAL PROGRESSION SCALE FOR CLINICAL STUDIES OF COVID-19

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

APPENDIX E: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	3	14/06/2022	Brama Hanumunthadu/Nelly Owino/Maheshi Ramasamy	• Edit to Typhoid / Paratyphoid vaccine as an exclusion criteria • Clarification of SARS-COV-2 test as a temporary exclusion criteria • Addition / modification of mailout language and inclusion of use of GP databases to identify potential participants
NSA01	3.1	13/01/2023	Timothy Crocker-Buque	• The protocol has been has been edited to clarify: • Minor edit to Participant study windows for the V5, V8 and V11 (D28 post vaccination Visit). Edit changed from D28 +/- 4 days to +/- 7 days. This change has been made to improve participant management and Data collection.

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC / MHRA / HRA submission.
Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, HRA (where required) or MHRA.

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Sal*monella* **V**accine Study in **O**xford

SALVO

INFORMED CONSENT FORM

Participant's Name: _____

Participant Initials: _____

Participant Number: S A L ___ ___ ___

If you agree, please initial box:

Section 1: Study Procedures	
1. I confirm that I have read the information sheet dated..... (version.....) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2. I have spoken with Dr/Nurse_____	
3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	
4. I have received detailed information about the intervention schedule, study procedures, potential side effects and their importance.	
5. I agree to be randomised to receive either the iNTS-GMMA vaccine (Lower or Full Dose Schedule) or the placebo schedule as detailed in the participant information sheet and I am aware of the risks and side effects associated with each intervention. I am aware that each schedule includes 3 vaccine or placebo administrations.	

6. I will bring the 24-hour contact reply slip, signed by my 24-hour contact prior to receiving the first dose of vaccine or placebo. I agree that the study team may contact this person if I cannot be contacted during the study.	
7. I agree to refrain from donating blood/blood products for the duration of the study.	
8. ***Women only***: I understand the need to ensure that I or my partner use effective contraception one month prior to first vaccination and continue to do so for the remainder of the study.	
Section 2: Personal Information	
9. I agree to OVG storing and using my personal information as described in the information booklet.	
10. I agree to my General Practitioner being informed of my participation in this study. I agree to my GP and/or other treating doctors being approached for additional information regarding my medical and vaccination history and study staff to access my NHS medical records either via my GP or the electronic patient records system.	
11. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University of Oxford (Sponsor), from regulatory authorities [and from the NHS Trust(s)], where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. I agree to my National Insurance (if UK citizen) or Passport number being used to register me on TOPS. I understand that it will be stored electronically for the duration of the study.	
12. I understand TOPS is a Health Research Authority database that aims to prevent healthy volunteers from taking part in too many studies. I understand that only staff at OVG and other research units can use the database and OVG may call other units, or OVG may be called, to check volunteer details.	
13. I agree to provide my bank account details including my account name, sort code and account number for reimbursement purposes. I understand that my banking details will be stored electronically as described in the information booklet. I understand that my personal information will be shared to the extent required to process or verify eligibility of payments as described in the information booklet.	
Section 3: Research Samples and Data	
14. I agree to donate blood and saliva samples. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	

15. I agree to my de-identified data and biological samples being sent and stored within and outside of the European Union for analysis by collaborating research groups as described in the information booklet.		
16. I understand and agree that some of my samples will be used to investigate the genetic factors determining the response to iNTS-GMMA vaccine or placebo.		
If all of the applicable sentences above are initialled, meaning "yes", then please continue:		
17. I agree to take part in this study.		
Optional:		
18. I agree to donate stool samples for this study. I consider these samples a gift to the University of Oxford and I understand I will not gain any direct personal or financial benefit from them.	Yes	No
19. I agree to be contacted about ethically approved research studies for which I may be suitable. I understand that agreeing to be contacted does not oblige me to participate in any further studies.	Yes	No

_____	_____	_____
Name of Participant	*Date*	*Signature*
_____	_____	_____
Name of Person taking Consent	*Date*	*Signature*

**1 copy for participant; 1 original for researcher site file.*

OXFORD VACCINE GROUP

Sal*monella* **V**accine Study in **O**xford
SALVO

PARTICIPANT INFORMATION SHEET

You are invited to take part in a study to test a new vaccine against Invasive Non-Typhoidal *Salmonella* (iNTS), an important cause of blood poisoning in children and adults in sub-Saharan Africa. The study is being run by the Oxford Vaccine Group, which is part of the University of Oxford.

Participation in the study is entirely voluntary. Before you decide whether to take part, it is important for you to understand what the study is about and what participation would involve. Please take time to read the information carefully and discuss with others if you wish. If anything is unclear or you would like further information, please contact the study team.

Thank you for taking the time to consider taking part in this study.

Contact Details
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Email: info@ovg.ox.ac.uk
Website: www.ovg.ox.ac.uk

Who are the Oxford Vaccine Group?

The Oxford Vaccine Group, which is part of the **University of Oxford**, is an independent research team of doctors, nurses and play assistants. We carry out research studies of new and improved vaccines for babies, young children, teenagers and adults, and teach doctors and nurses about immunisations. In the past 5 years alone, over 7,000 participants in the Thames Valley area have taken part in our research studies.

What is invasive Non-Typhoidal *Salmonella* disease?

Non-typhoidal *Salmonella*e are a group of bacteria, that are well known to cause food poisoning throughout the world. However, in certain circumstances they can cause a more serious disease, where *Salmonella* can spread beyond the gut leading to blood poisoning, and in some cases sepsis and death. This is called invasive non-typhoidal *Salmonella* (iNTS) disease and is of particular concern in individuals with a weaker immune system. iNTS disease occurs in over half a million people a year, and particularly affects children under 5 years of age in sub-Saharan Africa, where it causes significant disease with over 200,000 cases and 31,000 deaths per year. Adults can also be affected by iNTS disease, particularly those with HIV, malaria or malnutrition.

What is the purpose of the study?

In this study we are investigating a new vaccine against iNTS called the iNTS-GMMA vaccine. This new vaccine is developed by the GSK Vaccine Institute for Global Health (GVGH), a GlaxoSmithKline (GSK) company based in Italy with which the Oxford Vaccine Group is collaborating for the development of a vaccine against iNTS. This will be the first time this vaccine will be given to human volunteers. It contains small amounts / particles of the outer surface of the two most common bacteria that cause iNTS disease (*Salmonella* Enteritidis and *Salmonella* Typhimurium). These particles previously known as Generalised Modules for Membrane and Antigens', and currently abbreviated to 'GMMAs', constitute the main component of the vaccine. **The vaccine does not contain *Salmonella* bacteria and therefore cannot cause infection or disease**. It is hoped that these GMMA particles can stimulate the immune system to produce a protective response against iNTS bacteria and thus prevent future blood stream infections by these bacteria. The GMMA particles are diluted in Alhydrogel, a common vaccine component designed to reduce local side effects. The study is being conducted to evaluate the safety of the vaccine and how well it stimulates the immune system against iNTS.

Not everyone will receive the active vaccine, some individuals will receive a placebo (non-active comparison). The placebo contains all the components of the iNTS-GMMA vaccine except for the active GMMA particles i.e. Alhydrogel alone and cannot stimulate the immune system to produce a protective response against iNTS bacteria. Participants will be enrolled sequentially into 3 groups and randomly allocated to receive either the iNTS-GMMA vaccine or the placebo. Randomization means that neither you nor your doctor will choose whether you receive the active vaccine or placebo. In this study, a computer will assign this to individual participants, like flipping a coin. You have an equal chance of receiving the vaccine versus placebo in groups 1 and 2 and have more chances of receiving the vaccine than the placebo in group 3.

Why have I been invited?

We are inviting healthy adults aged 18 to 55 years old to take part in this study. We would not want to recruit anyone who has significant health issues, anyone with altered immune function or any females who are pregnant or thinking of starting a family soon. We use various ways to contact potential volunteers, including the Electoral Roll and the National Health Applications and Infrastructure Services (NHAIS) who hold the central NHS patient database (Open Exeter). This database identifies all persons within the local area who are in the appropriate age range. Whilst we do commission the invitation to take part in the study, we do not have access to personal data and are not directly responsible for the mail out. In addition, you may have previously expressed an interest in taking part in studies at the Oxford Vaccine Group.

What should I consider?

We are keen to recruit healthy volunteers who are:

- Willing to take part and able to attend all study visits
- Aged between 18 and 55 years old, inclusive
- Willing to allow us to communicate with their GP to notify them of your participation in the study and to check your medical history
- (Females) Willing to use effective contraception from 1 month prior to vaccination and for the remainder of the study

Do I have to take part?

No. We are looking for volunteers. Should you volunteer and later change your mind (for whatever reason) it is your right to do so, and you would not need to provide an explanation to the study team or anyone else. In addition, your decision to withdraw would not affect any ongoing medical care you are or will be receiving.

Whatever you choose it's important that you are happy with your decision and it is not the role of the study team to decide for you. If you choose to withdraw after the receiving the vaccine, we would stop all research related activities. However, we would like to check that you remain well after receiving the vaccine for your own safety which may or may not include follow up visits / blood tests. We would use the samples and data we have collected from you in our analysis of the study, up until the point you informed us that you wanted to withdraw.

What are the possible benefits of taking part?

There are no clear benefits to you if you take part in this study. However, you would have the knowledge that you played a part in the early stages of developing a new vaccine against a bacteria that causes a significant burden of death and disease, particularly in sub-Saharan Africa and in children under 5 years of age for which there are currently no licensed vaccine.

Are there any possible disadvantages or risks from taking part?

In general, the risks are in relation to the vaccine/placebo, blood and oral fluid sampling. In addition, you would be asked to attend regular visits at the CCVTM. In regards to COVID-19 please see the COVID-19 section below.

- **Vaccine**

General Vaccines

Intra-muscular vaccination can commonly cause reactions, although most tend to be minor and only last a few days. These may typically include injection site - discomfort, redness, and swelling. As for all vaccines some volunteers occasionally may feel generally unwell, develop fevers, muscle aches, joint aches, headache, experience loss of appetite, nausea / vomiting, abdominal pain or diarrhoea. Not everyone will experience symptoms and if they do occur, they should resolve after a few days.

Anaphylaxis is a very rare but a potentially life-threatening allergic reaction and may occur after immunisation. All clinical staff are trained in the immediate treatment of anaphylactic reactions including the use of intra-muscular adrenaline. It is for this reason you need to wait at least 60 minutes after each vaccine dose is given, as this would be within the typical time frame should this reaction occur.

iNTS GMMA Vaccine

This study is the first time that the iNTS-GMMA vaccine will be given to human participants. The studies performed in animals prior to moving on to human trials have shown good safety results. In addition, GMMA-based vaccines against other bacteria have been safely used in over 190 volunteers. These vaccines were found to be well-tolerated and safe in the volunteers who received them. Nevertheless, this is a new vaccine and there may be side effects we do not know about. It is important for you to be aware of this. However, we have multiple measures in place to ensure your safety during the trial as outlined below.

Throughout the study, the safety of the participants in all groups will be monitored following vaccination. This will be done by reviewing of symptoms at visits and through the electronic Diary (eDiary). For your safety there will be an independent safety committee consisting of an independent panel of experts known as the Data and Safety Monitoring Committee (DSMC) who will know whether you have received vaccine or placebo in order to periodically monitor the overall safety of the trial. This committee will review the safety data particularly as the trial proceeds between Groups 1, 2 and 3 and would be required to approve progression from lower to full dose active iNTS-GMMA vaccine within the trial. Should there be any concerns this committee has the power to halt the trial for further evaluation. In addition, you will be provided with study team contact details who are available 24/7 should you need to contact us.

We would ask you to provide contact details of a person who would act as a second contact. Only to be used in an emergency or needing to contact you urgently.

- **Placebo**

The placebo (Alhydrogel) is a common component used in many vaccines and would not usually cause any side effects other than the ones known for any general vaccine as listed

above. Not everyone will experience side effects and if they do occur, they should resolve after a few days.

- **Blood/Oral Fluid Sampling**

Blood tests can be painful and sometimes leave bruising and/or temporary discomfort, but these all resolve in a very short period of time. Rarely fainting can occur. Oral fluid samples are collected with a cotton swab of the mouth and should not cause any discomfort.

- **Pregnancy**

For females, you should not take part in this trial if you are pregnant or breastfeeding. It is currently unknown whether the vaccine being tested is safe during pregnancy. For this reason, it is important that women use adequate contraception during the study period. Women who are not of childbearing potential (i.e. postmenopausal or permanently sterile due to surgery such as a hysterectomy) will not be required to use contraception. This will be discussed with you at the screening visit. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the trial, although we will ask to follow you up for safety reasons.

Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible.

- **COVID-19**

It is difficult to predict the time course of the COVID-19 pandemic. Should further peaks occur during the study, we will implement specific measures to ensure your safety whilst taking part in the study. **The safety of our study participants remains the prime consideration during the trial.** If you have not already received a COVID-19 vaccine and you become eligible (according to UK policy) for the vaccine during the study, we would talk to you about this. If you agreed and if it was possible, we would find a mutually agreeable time for you to receive this vaccination. We would not prevent you from receiving this vaccination. During the study we will follow up-to-date government advice including on advising testing, self-isolation, and personal protective equipment (PPE) as necessary. Should you develop symptoms of COVID-19 or test positive for COVID-19 during the study, we would want to know about this prior to coming to clinic. This may affect the timings of your clinic visits and we would want to assess whether it is safe for you to receive further study vaccinations.

What will happen to me if I decide to take part?

- **Recruitment**

If you express an interest in taking part, a member of the Oxford Vaccine Group will contact you by telephone to discuss the study and answer any questions you may have. If you have accessed the participant information sheet via the online questionnaire you will have already answered initial eligibility questions. We would like to ask you a few more detailed questions to assess your eligibility.

Following this, if you are interested and seem suitable for the study then we would arrange for you to come to our clinic for a screening visit. In addition we would send you a consent

form (paper or electronic) giving permission for the study team to access your medical records to obtain this information via the electronic patient records or through your GP. We would then ask you to return a copy of the signed consent form (paper or electronic). A countersigned form will be provided at the screening visit. This consent form is only to allow access to your medical records, and not the consent for enrolment on to the study. If you choose to participate in the study a separate consent will be taken for inclusion into the trial.

- **Screening Visit**

The **purpose of screening tests is to ensure that you are eligible to take part** and that by taking part in the study you are not taking on any extra risk to your health. At the screening visit we would sit with you and go through the study in detail. This visit would provide an opportunity for you to ask any questions you might have about the study and what's involved. You would be allowed as much time as you feel necessary before making any decision on whether to take part.

Is coming to screening a commitment to taking part?

No. It's an opportunity to meet with the study staff and ask questions; you do not need to make a decision there and then.

What if I wish to volunteer?

If you are keen to proceed, we would ask you to sign an **informed consent form**. Only once this is signed would we then start any study procedures.

What are the study procedures at the screening visit, if I decide to volunteer?

We would ask you questions about your health, undertake a physical examination and take a blood sample (of 10ml; approximately two tablespoons). We would also require a urine sample. Blood testing includes HIV and hepatitis B and C tests, as well as screening for anaemia, liver and kidney function. For females, we would perform a pregnancy test on your urine sample.

Demographic data including your name, date of birth, gender, ethnicity and contact details will be collected, if not already recorded at the recruitment stage. Data protection regulation governs how we store and process your data. Please see 'What will happen to my data?' below for further information.

We would also seek your consent to register your name on the 'The Over-volunteering Prevention System' (TOPS) national database. This is designed to guard against the potential for harm that can result from excessive volunteering in clinical trials involving investigational medicinal products and blood donations. This would be done using your National Insurance number or passport number, and all information is kept confidential. More information can be found at http://www.hra.nhs.uk/about-the-hra/our-committees/the-over-volunteering-prevention-system/. If we have not already done so, prior to your screening visit we would also ask for your consent to access your medical records via the electronic patient record system or via your GP to obtain any relevant medical history that may affect your participation in the study. Once the study team have confirmed your suitability for the trial, we would inform you and arrange a date for your

first visit. You would be formally enrolled into the study at the time the first vaccine dose is given.

- **Enrolment**

If you are eligible for the study, you will be enrolled into 1 of 3 groups (outlined below) dependant on your enrolment date:

Group 1	Randomly allocated 1:1 to receive either the lower dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 2	Randomly allocated 1:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to twelve participants will be allocated to this group.
Group 3	Randomly allocated 2:1 to receive either the full dose iNTS-GMMA vaccine or placebo. Up to eighteen participants will be allocated to this group.

Table 1: Enrolment of Groups 1-3

- **Overview of Study Visits**

Each participant would receive 3 vaccinations with the iNTS-GMMA vaccine or placebo at intervals of 0, 2 and 6 months. In addition, each participant would require blood tests immediately before each vaccination and at specific intervals after each vaccination. The study will require a total of 12 visits over a 12-month period.

A simplified overview of the study is shown in the diagram below:

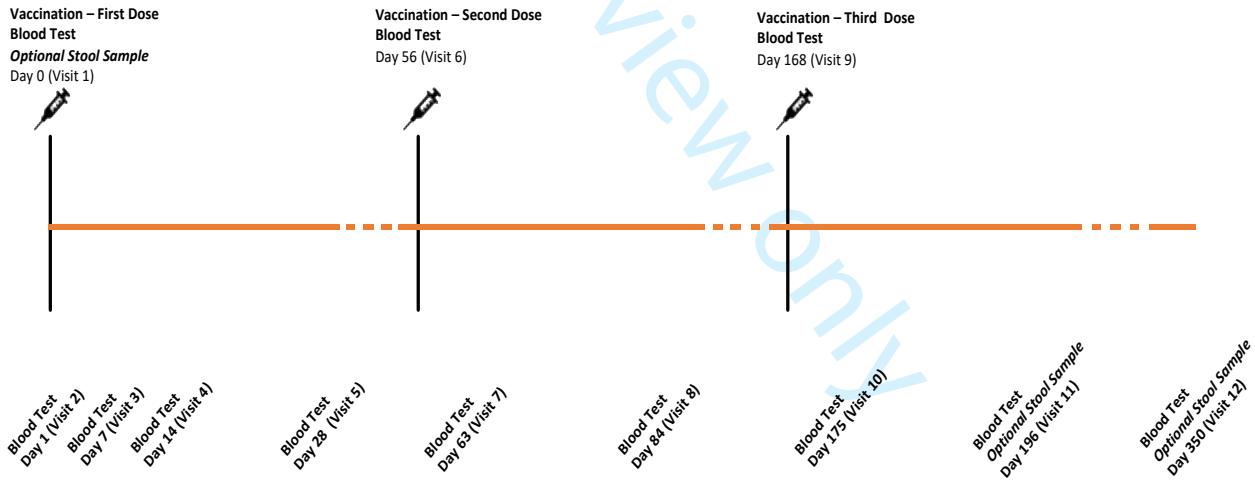

Figure 1: Overview of Study Design

Neither you nor the study team analysing the data will know whether you have received the iNTS-GMMA vaccine or the placebo. This is termed a 'participant-observer blind' trial and provides greater confidence in the final conclusion of the study.

*For participants in group 3 we will collect an additional blood sample (30mls, equivalent to 6 tablespoons) to develop a serum standard at Visit 8 (Day 84). The purpose of the serum

standard is to create a supply of antibodies directed towards the iNTS-GMMA Vaccine that can be used as a reference standard to develop laboratory tests and compare how well this vaccine or future vaccines are working in the individuals being vaccinated. Due to the study design participants who receive the placebo will also donate this additional sample. While these samples will undergo the same laboratory tests, these samples will not be used as a reference standard but may be used in laboratory test development.

- **Vaccine Visits (Visit 1, 6 and 9)**

You would be given a date, time and place to come to the Oxford Vaccine Group at the Churchill Hospital. We would start by checking that you are happy to remain in the study and ask if anything has changed medically since we last saw you.

We would check your pulse and blood pressure at every visit, and for all women a pregnancy test would be done prior to administering each dose of vaccine or placebo. We would take blood and oral samples and give the first vaccine by injection into the muscle of the upper arm.

After vaccination you would need to wait with us for 60 minutes before leaving. This is standard practice to monitor any reactions to the vaccine.

We would give you a tape measure, thermometer and set you up on the **electronic diary** for you to record any symptoms or side effects and daily temperatures for the next **7 days following vaccination**. We would explain how you record this information when you are with us, and it should be entered electronically via a secure link and encrypted transfer on our website wherever possible. Monitoring the ediary will allow the team of research doctors and nurses to check on your wellbeing and ensure your safety.

- **Follow-up Visits**

There are two to four scheduled follow up visits following each vaccination. These visits usually last approximately 30 minutes. We would check on your progress during the trial, review any ediary entries, take recordings of your pulse and blood pressure and collect any blood or oral fluid samples as per the sample schedule below.

- **Samples**

We collect a blood sample at each visit. The amount will be between 5mL (about 1 tablespoon) and 70.5 mL (quarter of a cup) depending on the tests that will be done. The total amount of blood taken during the whole study (1 year) would be up to 667.5mL (about 3 cups), which is significantly less than the maximum amount of blood that could be donated over a year to the UK Blood Donation Service. This is in line with the blood donation guidelines and your body would replace this naturally after about three months. Repeated blood tests can cause anaemia (low haemoglobin), however we have checks and processes in place to ensure this is minimised, including reducing the blood volume we take from you. In rare cases we may ask you to see your general practitioner for a medical review. These blood tests are important to measure how good the vaccine is at producing an immunity to iNTS. In addition, we periodically check your general blood tests including full blood count, urea and electrolytes and liver function tests to ensure they are normal throughout the study.

At some visits we will collect an oral fluid sample using a simple 10 swab (1-2 minutes). In addition, to blood we are testing oral fluid to measure indicators of immunity to iNTS.

In addition, stool samples may be collected during the study. This is entirely **optional**. If you do not agree to the collection of stool samples, this will not affect your participation in the study. If you opt-in, the stool samples will be collected before the first vaccine is administered (Visit 1), 28 days after the third vaccine (Visit 11) and at the end of the study (Visit 12). We will test for the microscopic organisms such as bacteria, parasites and fungi that naturally occupy your gut to see if the mix of these microscopic organisms influences your response to the study vaccine. In addition we will test for the development of gut antibodies to Non-typhoidal *Salmonella*e. You will be given collection materials and the study team will explain how to collect the samples. We will receive the samples at the specified visits. However, if you are unable to provide a sample there is an option to free post if you are happy to do so. You will be given 'By Post' collection materials and the study team will explain how to collect and post the sample.

There might be times when your blood needs to be retested or a urine sample taken to confirm test results. This might happen at your next visit or at an unscheduled visit that you would need to come to the clinic for. At this visit the study staff would take a sample of your blood (usually 10 mL, 2 tablespoons) and collect a urine sample, as needed.

What will happen to the samples I give?

Your sample will be assigned a code and your data will also be identified only by this number. The material given to researchers will not have information that directly identifies you. The blood, urine, stool and oral fluid samples collected during this study would be analysed in the Oxford University Hospitals, University of Oxford research laboratories, while some blood samples will be shipped to the GSK Vaccine Institute for Global Health (GVGH), Siena, Italy or delegated laboratories based in Italy. We would also send some samples to other researchers working with us on this research project, including researchers outside the European Union. Your samples will be identified by a code instead of your name and the link between your name and the code number will not be shared with external companies and laboratories.

If you choose to take part in this study, we will be asking for your separate permission to store blood (including cells and DNA) samples, in a collection of samples called Biobank. Details of this will be provided in a separate booklet after you are enrolled into this study, and you are free to say no to the Biobank and continue to take part in this study if you wish. If you do not wish for your samples to be stored in the Biobank, they will be destroyed 12 months after the last participant has completed the study.

What if any of my test results were abnormal?

If there are any abnormal results or undiagnosed conditions found in the course of the study these would be discussed with you and, if you agreed, your GP would be informed of these results. We would not report them to anyone else without your permission. For example, a new diagnosis of high blood pressure might be made. Any newly diagnosed conditions would be looked after by your GP.

Reimbursement

There are no costs for you to participate in this study. Study participants would be reimbursed for their time, travel and inconvenience of taking part in the study. The maximum reimbursement for any volunteer who completes the whole study is £585. All participants will be reimbursed based on the following figures:

Travel expenses: £15 per visit
Inconvenience of blood tests: £10 per blood donation
Time required for visits: £20 per visit

The sum reimbursed is on a pro rata basis, so, if for example, you choose to withdraw halfway through the study we would calculate your reimbursement based on the visits you have attended and samples that have been obtained.

Payments are made directly by bank transfer in instalments during the study. For this reason, we would require participants to provide their bank details at screening. Bank details would be kept confidential. Personal information such as your name, bank details and national insurance number may be shared with the University finance team to process or verify your reimbursement payments. Financial auditors may also audit the records where this information is held. All confidential data will be stored according to the UK General Data Protection Regulation (see below).

You may also receive reimbursement for any unscheduled visits you attend (if you have symptoms from the vaccine and need to be assessed). You would be reimbursed £45 per unscheduled visit, up to a maximum of £135 (equivalent of 3 unscheduled visits). If you do not require any unscheduled visits, you will not be reimbursed for this amount.

Would my taking part in this study be kept confidential?

Yes. All information that is collected about you during the course of the research would be coded with a study number and kept strictly confidential. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. You can search this website at any time. The website will include a summary of the research study results, but it may be many years before research results are posted. Any information about you that leaves the clinic would have any identifiable information removed so that you could not be recognized, with the exception of letters sent to your own GP. In order to enrol into this study, you would be required to sign a form, documenting that you consent for us to contact your GP. This is to inform him/her that you would be entering the study, and to ensure there are no medical reasons that would prevent you from taking part in this study. No one else would be told that you are involved in the study. We would only notify your GP of the results from any medical tests we performed with your permission.

Your information would be stored on a secure server, and paper notes would be held by the Oxford Vaccine Group in a locked cabinet. Your data is retained in case we need to contact you regarding any study related matters or if you wish to contact us regarding your participation in the study. We may also contact you to inform you of future related studies.

Responsible members of the University of Oxford and the Oxford University Hospitals NHS Foundation Trust may be given access to data for monitoring and/or audit of the study to ensure that the research is complying with applicable regulations. In addition, the following groups may inspect the study records without violating your confidentiality:

- Monitors who check that the study is being conducted to a high standard, including the Data and Safety Monitoring Committee (DSMC), an independent panel of experts responsible for trial safety and the Medicines and Healthcare Products Regulatory Agency (MHRA).

Coded data and samples would be sent to other researchers working with us on this research project, including researchers outside the European Union. Please note that your blood samples contain cells and DNA. Your DNA is unique to you so it can never be completely anonymous.

What will happen to my data?

Data protection regulation requires that we state the legal basis for processing information about you. In the case of research, this is 'a task in the public interest.' The University of Oxford is the data controller and is responsible for looking after your information and using it properly.

We will be using information from you and your medical records in order to undertake this trial and will use the minimum personally identifiable information possible. We will keep identifiable information about you such as contact details for a minimum of 5 years after the trial has finished. The need to store this information for longer in relation to licensing of the vaccine will be subject to ongoing review.

Paper notes will be held by the Oxford Vaccine Group in a locked cabinet. Once the trial has been completed, all documents, including personally identifiable data, would be archived in a secure facility, for a minimum of 5 years. Storage of this data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. If you complete online or telephone screening, and do not progress to in-person screening, your data will only be stored until the end of the trial.

If you have agreed that samples can be retained for future research then your personally identifiable information will be kept with restricted access solely for the purposes of sample management for a minimum of five years after the last sample has been either used or disposed of in order to meet regulatory requirements. Samples will be provided for future research only in a form that does not identifies you. We store research data securely at the University of Oxford indefinitely following removal of identifiable information.

The trial team will use your name and contact details, to contact you about the clinical trial, and make sure that relevant information about the trial is recorded for your care, in relation to your health during the trial and to oversee the quality of the trial. At the completion of the trial, unless you consent otherwise (e.g. if you request to be informed of other trials),

your personal details will not be used to contact you other than exceptional circumstances concerning your safety.

If you consent to take part in another trial carried out by the Oxford Vaccine Centre, we will retain a copy of your consent form until such time as your details are removed from our database but will keep the consent form and your details separate. Personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

Your bank details will be stored for 7 years in line with university financial policy.
Data protection regulation provides you with control over your personal data and how it is used. When you agree to your information being used in research, however, some of those rights may be limited in order for the research to be reliable and accurate. Professor Andrew J Pollard, or his successor, as Director of the Oxford Vaccine Group will have the responsibility for custody of the data.

Further information about your rights with respect to your personal data is available at:
https://compliance.web.ox.ac.uk/individual-rights

If you withdraw from the trial, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible.

You can find out more about how we use your information by contacting Oxford Vaccine Group on 01865 611400 or email info@ovg.ox.ac.uk.

What will happen at the end of the research study?

The results of the research will be published in a scientific medical journal and potentially presented at future conferences; this can potentially take a few years. All OVG publications will appear on the OVG website and you will receive a letter containing these results. Your individual results would not be identifiable, nor would you be identified in any report or publication. The results of the research will also potentially be used for future academic research within the Oxford Vaccine Group. Some of the research being undertaken will also contribute to the fulfilment of an educational requirement (e.g. a doctoral thesis).
Once the last laboratory test is performed in the study, all samples will be destroyed, unless you have consented for them to be transferred to the Biobank. If your samples are going to the Biobank, a copy of your informed consent form (which contains your personal information), are stored with those samples.

What if there is a problem?

If you have private medical insurance, you are advised to contact your insurance company before participating in this trial. The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this study.

Where can I get advice on whether to take part?

We are happy to answer any questions you might have and contacting us does not commit you to taking part in the study.

Other useful links for general information on taking part in research include:

- www.crn.nihr.ac.uk/can-help/patients-carers-public/how-to-take-part-in-a-study/
- www.nhs.uk/Conditions/Clinical-trials/Pages/Introduction.aspx

What if I wish to complain?

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Professor Andrew Pollard, Director of the Oxford Vaccine Group, (Tel: 01865 611400, Email: info@ovg.ox.ac.uk) or you may contact the University of Oxford Research Governance, Ethics and Assurance (RGEA) office on 01865 (6)16480 or the Head of RGEA, email ctrg@admin.ox.ac.uk.

At any time during the study you would be entirely free to change your mind about taking part, and to withdraw from the study. This would not affect your subsequent medical care in any way.

Who is funding the study?

The study is funded by an EU Framework Programme for Research and Innovation, Horizon2020, Vacc-iNTS no 815439 grant, as part of a wider project to progress the iNTS-GMMA vaccine initially through the SALVO clinical trial with a further study to take place in sub-Saharan Africa. For further information on the Vacc-iNTS project please see: https://vacc-ints.eu

Independent monitoring of the study will be undertaken by Appledown Clinical Research Ltd which will be funded by GSK Vaccine Institute for Global Health (GVGH).

Who has reviewed and approved this study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' interests. This study has been reviewed and given a favorable opinion by South Central - Oxford A Research Ethics Committee. In addition, this study has been reviewed by the Medicines and Healthcare Regulatory Agency (MHRA) the UK agency responsible for ensuring that medical products under investigation (in this case the active vaccine) is safe and appropriate to continue to clinical trial.

In summary, what would happen if I decide to take part in the study?

- We would ring you to check it is appropriate to include you in the study.
- You would then attend a screening visit in our department (CCVTM).
- At the screening visit we would go through the study in detail and answer any questions you may have. If you are happy to proceed, we would then ask you to sign a consent form. You would then have a brief medical assessment including a medical

history and physical examination. You would have a blood test and urine test (and a pregnancy test for women). These are to assess your eligibility for the study.

- Following satisfactory screening results confirming your eligibility we would invite you to the initial vaccination visit (V1) and enroll you into the study.
- You would be vaccinated with the study vaccine or placebo, once enrolled, then at month two and six.
- You would be seen two to four times after each vaccination; with a final visit one year after the first vaccination. These will include a blood test +/- an oral fluid sample.
- You would be required to fill in an eDiary for seven days after each vaccine.
- The study duration is approximately one year at which time you will be seen 12 times at the CCVTM (not including unscheduled visits or the initial screening visit).

What do I do now?

Thank you for considering taking part in this study. You do not need to make a final decision straight away. If you wish to discuss any element of the study further, then please contact us by either

- telephone **01865 611400**
- website: http://trials.ovg.ox.ac.uk/trials/salvo
- email: info@ovg.ox.ac.uk

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supp Material – Trial Summary
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Supp Material

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	Supp Material
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Supp Material
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Supp Material
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	5

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	Supp Material
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	Supp Material
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5 Supp Material
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Supp Material

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6 Supp Material In Blinding and Randomisation Plan
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Supp Material In Blinding and Randomisation Plan
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Supp Material In Blinding and Randomisation Plan
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Supp Material In Blinding and Randomisation Plan
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Supp Material In Blinding and Randomisation Plan
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	Supp Material

		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Supp Material
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Supp Material
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8 Supp Material
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	8 Supp Material
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	8 Supp Material

Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Supp Material
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Supp Material
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Supp Material
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	8
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Supp Material
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Supp Material
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Supp Material
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Supp Material
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	Supp Material

Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Supp Material
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Supp Material
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	8
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA – no intended use of professional writers
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Protocol – as part of submission
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supp Material
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp Material