Clinical Study Protocol
Sponsor:
GlaxoSmithKline Biologicals SA
Rue de l'Institut 89,
1330, Rixensart, Belgium

Primary Study vaccine and number	• GlaxoSmithKline (GSK) Vaccines Institute for Global Health (GVGH) *Shigella sonnei* GMMA vaccine (GVGH *S. sonnei* 1790GAHB vaccine), property of GSK Biologicals.
Other Study products	• GAHB-Placebo (control and diluent in the bedside mixing). • *Shigella sonnei* 53G (challenge strain developed by the Walter Reed Army Institute of Research [WRAIR], Silver Spring, Maryland, USA) referred to as the challenge agent.
eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Investigational New Drug (IND) number	IND18163
Date of protocol	Final: 12 December 2017
Date of protocol amendment	Amendment 1 Final: 08 February 2018 Amendment 2 Final: 22 June 2019 Amendment 3 Final: 07 September 2019 Amendment 4 Final: 19 February 2019 Amendment 5 Final: 15 April 2019 Amendment 6 Final: 23 July 2019
Title	Efficacy, safety and immunogenicity of GVGH *Shigella sonnei* vaccine (1790GAHB) in a human challenge study of healthy non-immune adults.
Detailed Title	A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population.
Coordinating authors	PPD, PPD, Scientific Writers (XPE Pharma & Science for GSK Biologicals)

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Contributing authors	• PPD, GVGH Head of Clinical Development and Regulatory Affairs • PPD, Project Physician • PPD, PPD, Biostatisticians • PPD, GVGH Clinical Research Associate • PPD, GVGH Head of Clinical Operations • PPD, Clinical Laboratory Sciences Read-out Team Leader • PPD, PPD, PPD, Clinical Laboratory Sciences Study Managers • PPD, PPD, GVGH Serology Representatives (Scientific Technical Leaders) • PPD, PPD, Safety Physician, Safety Evaluation and Risk Management team • PPD, Safety Scientist, Safety Evaluation and Risk Management team • PPD, Oversight Data Manager • PPD, GVGH Head of Regulatory Affairs • PPD, Global Patent Affairs Manager

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Protocol Amendment 6 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
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Date of protocol amendment	Amendment 6 Final: 23 July 2019
Detailed Title	A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population
Sponsor signatory	Audino Pietro Mario Podda, GVGH Head of Clinical Development and Regulatory Affairs
Signature	
Date	

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Protocol Amendment 6 Rationale

Amendment number:	Amendment 6

Rationale/background for changes:

- To add an additional interim analysis for immunogenicity data, which will allow a faster interpretation of study results and planning of subsequent follow-up studies.
- To correct an error in the definitions of dysentery and more severe shigellosis, and align these definitions to other CHIM studies; oral temperature ≥ 38.0°C (fever) is added to the definitions.
- To correct an error in the primary completion date of the study.
- To clarify the different applicable case definitions of dysentery in the study.
- To provide clarification in the study design by showing the inpatient stay in the study design figure as per the Schedule of Activities table.
- To specify the allowed time interval window for the blood draw that occurs during the inpatient stay.
- To specify the assay used for the α4β7 plasmablast analysis as measuring both positive and negative cells by ELISA, and not using flow cytometry.
- To change the assay used for HLA-B27 testing from PCR to qualitative flow cytometry.

Protocol Amendment 6 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' study vaccine/placebo and challenge agent and other study-related duties and functions as described in the protocol and that bedside mixing at the study site will be performed according to GSK Biologicals instructions only by trained site staff with appropriate qualification required by local regulations.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccine, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
IND number	IND18163
Date of protocol amendment	Amendment 6 Final: 23 July 2019
Detailed Title	A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. **Sponsor**

GlaxoSmithKline Biologicals
Rue de l'Institut 89,
1330 Rixensart, Belgium

2. **Sponsor Medical Expert for the Study**

Refer to the local study contact information document.

3. **Sponsor Study Monitor**

Refer to the local study contact information document.

4. **Sponsor Study Contact for Reporting of a Serious Adverse Event**

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 9.4.2.

5. **GSK Biologicals' Central Safety Physician On-Call Contact information for Emergency Unblinding**

GSK Biologicals Central Safety Physician and Back-up Phone contact: refer to protocol Section 9.8.

SYNOPSIS

Detailed Title

A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population.

Indication

The targeted indication of the vaccine is the prevention of moderate to severe diarrheal disease caused by *Shigella sonnei* (*S. sonnei*).

Rationale for the study and study design

- **Rationale for the study**

GSK Vaccines Institute for Global Health (GVGH) has developed a vaccine against *S. sonnei* (1790GAHB vaccine) using the new Generalized Modules for Membrane Antigens (GMMA) platform technology.

The study vaccine could be the stepping stone for the development of a multivalent broadly protective Shigella vaccine for vaccination of impoverished communities where shigellosis is endemic. However, a standalone monovalent vaccine against *S. sonnei* could be used to protect travelers against diarrheal shigellosis, as the vast majority of travelers' shigellosis is caused by *S. sonnei*, and even to protect infants in endemic regions where shigellosis is primarily caused by *S. sonnei*.

The 1790GAHB vaccine has been tested in two Phase I dose escalation studies in Europe to assess its safety and immunogenicity via three routes of administration: intramuscular (IM), intranasal (IN) and intradermal (ID). The results from the first study (dose escalation with IM vaccination) have shown that the vaccine has an acceptable safety profile and is well-tolerated up to a dose of 100 µg. Additionally, the serologic assessment showed that one dose of ≥ 1.5/25 µg OAg/protein of 1790GAHB vaccine elicits serum immunoglobulin G (IgG) anti-*S sonnei* lipopolysaccharide (LPS) levels ≥ 121 enzyme-linked immunosorbent assay (ELISA) units/mL (EU/mL) (i.e., median antibody titer in a panel of 87 Israeli convalescent subjects after natural infection by *S. sonnei*). The results from the second study (dose escalation with ID, IN and IM vaccination) showed that 1790GAHB vaccine is well-tolerated also when administered by the ID and IN routes of vaccination. However, immunogenicity data have shown that 1790GAHB vaccine administered by the ID and IN routes is not as immunogenic as 1790GAHB vaccine administered by the IM route.

Therefore, it has been decided to proceed with the clinical development program of this vaccine only using the IM vaccination route. In terms of dosage, the regimen tested in Phase I studies (three doses given one month apart) did not show any significant benefit from the third dose in terms of immunogenicity, therefore a two dose schedule was selected for next studies. A Phase IIa study, conducted in endemic regions of Africa (i.e., Kenya), has just been completed and confirmed the acceptable safety profile and immunogenicity of 1790GAHB vaccine.

Performing a vaccine-human challenge study may give the opportunity to establish evidence of clinical protection induced by the candidate *S. sonnei* vaccine (1790GAHB vaccine) at an early development stage.

- **Rationale for the study design**

A human challenge model of *S. sonnei* disease for vaccine testing was established in Thailand [Bodhidatta, 2012] and showed an attack rate (AR) of 75% in non-immune adult volunteers receiving a challenge dose of 1680 colony forming units (CFU) of *S. sonnei* strain 53G. Clinical and laboratory endpoints of infection and disease, namely diarrhea, dysentery, fever and shedding of *S. sonnei* in stools were used to calculate the AR and thus to establish the optimal infective dose. In a subsequent study though [Pitisuttithum, 2016], the AR in the control group, using this model, was much less than previously published.

Given this variability, before starting a vaccine-challenge study, the proposed human challenge model of *S. sonnei* to be used has been appropriately developed and the challenge dose and AR reliably established by the Cincinnati Children's Hospital Medical Center (CCHMC) (Ohio, United States of America). The current GSK vaccine-human challenge study will be conducted at CCHMC and will use the developed challenge model. Volunteers will be treated after development of symptoms of shigellosis.

- **Rationale for the use of placebo**

As currently there is no widely available vaccine against shigellosis, the efficacy and safety of the 1790GAHB vaccine will be evaluated against a placebo. The placebo is used to better describe the safety profile of the *S. sonnei* vaccine as well as its efficacy in terms of clinical protection against shigellosis caused by *S. sonnei*.

Objectives

Primary

- To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo (reduction of shigellosis according to the protocol primary case definition, i.e. Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea, OR shedding with an oral temperature of ≥ 38.5°C after challenge with *S. sonnei* strain 53G).

  Criterion:

  Vaccine efficacy (VE) of 1790GAHB vaccine will be shown if the lower limit (LL) of the 90% confidence interval (CI) of VE will be above zero.

Secondary

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - Shigellosis as defined by the Controlled Human Infection Model (CHIM) expert working group on case definition.*
  - Shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].
  - More severe shigellosis, as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥ 38.0°C OR*** ≥1 severe constitutional/enteric symptom]]. ***(Amended: 23 July 2019)***
  - Shedding of *S. sonnei* strain 53G.
  - Severe diarrhea.
  - More severe diarrhea.
  - Dysentery.
  - Weight of all grade 3-5 stools.
  - Total number of all grade 3-5 stools.

- Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia).
- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- Time to onset of shigellosis according to the primary case definition after challenge.

*According to the working group, the participant must fulfil any one of the three following possible endpoints to qualify as having reached the CHIM case definition for this objective:

1. Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]
2. Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]
3. Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].

Safety

- To assess the safety and reactogenicity of the 1790GAHB vaccine in terms of solicited adverse events, unsolicited adverse events, serious adverse events (SAEs), adverse events of special interest (AESI) and laboratory parameters.
- To assess safety after challenge in terms of SAEs, unsolicited adverse events, AESI, and laboratory parameters.

Immunogenicity

- To evaluate the IgG ELISA immunogenicity profile of the 1790GAHB vaccine at 7 days and 28 days after the first and second vaccination (IgG ELISA coated with OAg containing LPS).
- To evaluate the immunogenicity profile of the 1790GAHB vaccine at pre-challenge and at 7 and 28 days after challenge (IgG ELISA coated with OAg containing LPS).
- Number and percentage of seroresponders for anti-*S. sonnei* LPS at 28 days after first and second vaccination.
- To assess anti- *S.sonnei* LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.

Tertiary

The following tertiary objectives, which are part of the study exploratory objectives may be evaluated in addition to the primary and secondary objectives and will complement assessment of the vaccine immunogenicity profile:

- Evaluate *S. sonnei* specific secretory immunoglobulin A ***(***sIgA***)*** in stool at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate ***S. Sonnei LPS IgG-specific*** α4β7***+/-*** plasmablast ***response*** at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64). ***(Amended: 23 July 2019)***
- Evaluate serum bactericidal activity against *S. sonnei* at Day 1 and 28 days after first and second vaccination.
- Evaluate correlation between serum anti-*S. sonnei* LPS IgG level, serum bactericidal activity titer, shigellosis, shedding, mild, moderate, severe or more severe diarrhea, and dysentery after challenge.
- Evaluate glycosylation of fragment crystallisable (Fc) portion of IgG antibodies at Day 1 and 28 days after second vaccination and association with clinical protection and/or serological endpoints.
- Evaluate inflammatory response from stools collected before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64).

- Evaluate gene expression signatures at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Investigate gut microbiome at Day 1 and 28 days after second vaccination and its potential association with protection and immune response.

Study design

- **Experimental design**: Phase IIb, observer-blind, randomized, placebo-controlled, mono-centric study with two parallel groups.
- **Duration of the study**:
  - Epoch 001: Starting at Screening Visit (Day -45 to Day -1) and ending before Visit 1 (Day 1).
  - Epoch 002: Starting the day of randomization and first vaccination (Visit 1) and ending before the receipt of the challenge agent, 28 days after second vaccination (Visit 5).
  - Epoch 003: Starting with the receipt of the challenge agent at 28 days after second vaccination (Visit 5) and ending 6 months after challenge (Visit 8).
- **Primary completion date (PCD):** Visit ***5*** (***Day 64***). ***(Amended: 23 July 2019)***
- **End of Study (EoS):** Last testing results released of samples collected for primary and secondary objectives at Visit 8.
- **Study groups:**

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min - Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
			Screening	1st and 2nd vaccination	Challenge
S. sonnei	36	18 years – 50 years	x	x	x
Placebo	36	18 years – 50 years	x	x	x

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/placebo/ challenge agent name	Study Groups	
		S. sonnei	Placebo
S. sonnei	1790GAHB	x	
Placebo	GAHB-Placebo		x
Challenge	*S. sonnei* 53G strain	x	x

- **Control:** Placebo control with GAHB-Placebo with the same composition as the vaccine except the active ingredient. GAHB-Placebo will also be used as diluent for bedside mixing of the vaccine.
- **Vaccination schedule:** Subjects will receive 2 doses of either the study vaccine or placebo 28 days apart. At 28 days after the second dose, all subjects will receive the challenge dose.
- **Treatment allocation:** Following the screening period before the first vaccination (Day -45 to Day -1), subjects will be randomized in a 1:1 ratio to receive either the study vaccine or the placebo.
- **Blinding:** The study will be observer-blind.

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Screening (Epoch 001)	N/A
1st and 2nd vaccination (Epoch 002)	observer-blind
Challenge (Epoch 003)	observer-blind

- **Sampling schedule:**
  - 5 mL for hematology: Screening Visit, Visit 2, Visit 3, Visit 4, Visit 5 and Visit 8. Additionally, hematology is repeated weekly until resolution if neutropenia occurs.
  - 5 mL for biochemical analysis: Screening Visit.
  - 10 mL for hepatitis B, hepatitis C and HIV: Screening Visit.
  - 10 mL for HLA-B27 testing: Screening Visit.
  - 5 mL for local anti *S. sonnei* LPS IgG Screening-ELISA: Screening Visit.
  - Urine sample for urinalysis: Screening Visit and Visit 5.
  - Urine sample for pregnancy test for women of childbearing potential: Screening Visit, Visit 1, Visit 3, Visit 5 and Visit 8.
  - 20 mL for immunological tests (antibody response) at Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, 7 days after Visit 5 (Day 64), Visit 6,

- For serum bactericidal activity tests at Visit 1, Visit 3 and Visit 5
- And for Fc glycosylation test on leftover serum samples collected at Visit 1 and Visit 5.
- 50 mL of peripheral venous blood for α4β7+/- plasmablast ***analysis by ELISA*** and RNA extraction from peripheral blood mononuclear cells (PBMC) for gene expression profiling: Visit 1, Visit 2, Visit 4 and 7 days after Visit 5 (Day 64). ***(Amended: 23 July 2019)***
- Stool sample for inflammatory response before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64).
- Stool sample: for microbiome testing at Visit 1 and Visit 5, for *S. sonnei* specific sIgA at Visit 1, Visit 2, Visit 4 and 7 days after challenge (Day 64) and stool analysis, culture and qPCR daily during inpatient stay (from Visit 5 to 7 days after challenge [Day 57 to Day 64] or prolonged until 2 consecutive negative stool samples if subject still shedding).

- **Type of study**: self-contained.
- **Data collection**: Standardized electronic Case Report Form (eCRF).
- **Safety monitoring:**

  An Independent Data Monitoring Committee (IDMC) will be established to monitor the safety of subjects throughout the study. The IDMC will review unblinded data, to assess safety signals and make recommendations to the Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects. The membership and roles of the IDMC will be outlined in the IDMC charter.

  The safety review team (SRT) will monitor the safety of subjects throughout the study. The SRT will review blinded data, assess safety signals and make recommendations to the IDMC and to the Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects.

A dedicated Safety Management Plan will be prepared to ensure appropriate safety reporting responsibilities and roles are defined.

Case definitions

Stools will be graded as follows: Grade 1: firm formed; Grade 2: soft formed; Grade 3: viscous opaque liquid or semi-liquid which assumes the shape of the bowl; Grade 4: watery opaque liquid; Grade 5: clear watery or mucoid liquid.

Diarrhea

For the purpose of this study, diarrhea is defined as:

- Moderate diarrhea: 4 to 5 loose or watery (Grade 3 to 5) stools or 400 to 800 grams of Grade 3 to 5 stools within 24 hours.
- Severe diarrhea: 6 or more loose or watery (Grade 3 to 5) stools or > 800 grams of Grades 3 to 5 stools within 24 hours or required medical intervention. In case of severe diarrhea, medical intervention is defined as intravenous (IV) fluids administration or anticipation of antibiotic treatment before the 5th day after challenge.
- More severe diarrhea: 10 or more loose or watery (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours.

Dysentery ***(Amended: 23 July 2019)***

For the purpose of the endpoint "Rate of shigellosis as defined by the CHIM expert working group occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]. In this case constitutional/enteric symptom are the following: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise.***

For the purpose of the endpoint "Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [≥1 reportable constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoint "Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]].", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoints "Dysentery" and "Confirmed S. sonnei 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia)", dysentery is defined as follows:

A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours and presence of constitutional/enteric symptoms. In this case, constitutional/enteric symptoms consist of headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting, and fever.

[Amended: 23 July 2019)

Shigellosis

For the primary and secondary objectives in the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea OR shedding with an oral temperature of ≥ 38.5°C.

For the secondary objectives in the challenge phase of the study, 2 definitions of shigellosis are used:

1. CHIM working group case definition for shigellosis as defined by any one of the three following possible endpoints:
   - Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]
   - Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]
   - Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].
2. Shigellosis as defined by any one of the three following possible endpoints:
   - Severe diarrhea defined as [≥6 loose stools in 24 hours OR >800 grams loose stools in 24 hours]
   - Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 moderate constitutional/enteric symptom].
   - Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom].

For the secondary objectives in the challenge phase of the study, more severe shigellosis is defined by any one of the two following possible endpoints:

- Moderate OR severe diarrhea [≥4 loose stools in 24 hours OR ≥400 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 severe constitutional/enteric symptom].

– Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom]. ***(Amended: 23 July 2019)***

Number of subjects

Target enrolment is approximately 72 subjects (36 per treatment group).

Endpoints

Primary

Efficacy

VE will be evaluated with:

- Rate of shigellosis (fulfilling the protocol primary case definition) occurring within a period starting with the challenge visit and lasting up to the end of the inpatient stay, in all subjects.

Secondary

Efficacy

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured during inpatient stay (Day 57 to Day 64) against:

- Rate of shigellosis ***as defined by*** the CHIM working group case definition for shigellosis) occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects. ***(Amended: 23 July 2019)***
- Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].
- Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥ 38.0°C OR*** ≥1 severe constitutional/enteric symptom]]. ***(Amended: 23 July 2019)***

- Shedding of *S. sonnei* strain 53G.

  Shedding of S. sonnei strain 53G is defined as positivity of at least one stool sample either by culture or qPCR or both.

- Severe diarrhea.
- More severe diarrhea.
- Dysentery.
- Weight of all grade 3-5 stools.
- Total number of all grade 3-5 stools.
- Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia)
- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- Time to onset of shigellosis after challenge, according to the protocol primary case definition.

Safety

Solicited adverse events

- Occurrence of each solicited local and systemic adverse event within 7 days after each vaccination.

Unsolicited adverse events

- Occurrence of unsolicited AEs within 28 days after vaccination and after challenge, according to Medical Dictionary for Regulatory Activities (MedDRA) classification.

Serious adverse events

- Occurrence of all SAEs and related SAEs from Day 1 to study end.

Adverse events of special interest

- Occurrence of AESI (i.e., symptomatic neutropenia) from Day 1 to study end.

Laboratory parameters

- Out of laboratory reference range and/or clinically significant value (according to local ranges) for hematology at 7 days after first and second vaccination and at last study visit (Visit 8).

Immunogenicity

The measures of immunogenicity, against the LPS of *S. sonnei*, will include:

- IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 7 and 28 days after first and second vaccination by antibody concentration at baseline (i.e., above vs. below the assay detection limit), as determined by anti-*S. sonnei* LPS IgG ELISA.
- IgG GMC pre-challenge (Day 57), 7 and 28 days after challenge (Day 64 and Day 85) as determined by anti-*S. sonnei* LPS IgG ELISA.
- Number and percentage of subjects achieving a post vaccination anti- *S. Sonnei* LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.
- Number and percentage of seroresponders* for anti-*S. sonnei* LPS at 28 days after first and second vaccination.

  *Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. For the purpose of this study seroresponse is defined as:

  – If the baseline value is greater than 50 EU/mL then an increase of at least 50% in the post-vaccination sample as compared to baseline.

  – If the baseline value is less or equal to 50 EU/mL then an increase of at least 25 EU/mL in the post-vaccination sample as compared to baseline.

Note: Threshold values/increases given in the definition of seroresponse might be subject to change during the course of the study (e.g., in case of optimization /qualification/validation of the anti *S. sonnei* LPS IgG ELISA).

Tertiary

- Specific anti-*S. sonnei* LPS sIgA antibody concentration in stool samples at Day 1, 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Frequency of *S. sonnei* LPS specific IgG α4β7+/- antibody secreting cells per 10^6 PBMC plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 8, Day 36 and Day 64). ***(Amended: 23 July 2019)***
- Glycosylation profiles of IgG antibodies at Day 1 and 28 days after second vaccination (Visit 5; Day 57).
- Number and percentage of subjects that show correlation between serum anti-*S. sonnei* LPS IgG concentration, serum bactericidal activity titer, shigellosis, shedding, moderate to severe diarrhea (MSD), dysentery and mild diarrhea after challenge.
- Concentration of stool markers of intestinal inflammation (e.g. calprotectin and myeloperoxidase) before oral challenge with S. sonnei, 53G at Visit 5 (Day 57) and daily during the inpatient stay after challenge administration (Day 57 to Day 64).
- The number of differential expressed genes 7 days after first and second vaccination and 7 days after challenge by comparing the relative abundance of mRNA sequences compared to Day 1 (pre-vaccination baseline).
- Diversity and frequency of microbiome components (taxa and/or genes according to technology) at Day 1 prior to vaccination and 28 days after second vaccination (Visit 5; Day 57).

Exploratory analysis for hypotheses generation will include the evaluation of the association of the microbiome components with efficacy and immunogenicity described above.

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LIST OF ABBREVIATIONS *(Amended: 23 July 2019)*

AE:	Adverse Event
AESI:	Adverse Event of Special Interest
AR:	Attack Rate
CCHMC:	Cincinnati Children's Hospital Medical Center (USA)
CDC:	Centers for Disease Control and Prevention
CFU:	Colony Forming Units
CHIM:	Controlled Human Infection Model
CI:	Confidence Interval
CMI:	Cell-Mediated Immunity
DNA:	Deoxyribonucleic acid
eCRF:	electronic Case Report Form
ELISA:	Enzyme-linked immunosorbent assay
EoS:	End of Study
EU:	ELISA unit
FAS:	Full Analysis Set
Fc:	Fragment crystallisable
FDA:	Food and Drug Administration, United States of America
GCP:	Good Clinical Practice
GMC:	Geometric Mean Concentration
GMMA:	Generalized Modules for Membrane Antigens
GMR:	Geometric Mean Ratio
GSK:	GlaxoSmithKline
GVGH:	GSK Vaccines Institute for Global Health
HCG:	Human Chorionic Gonadotropin

HIV:	Human Immunodeficiency Virus
HLA:	Human Leukocyte Antigen
IB:	Investigator Brochure
ICF:	Informed Consent Form
ICH:	International Council for Harmonisation
ID:	Intradermal
IDMC:	Independent Data Monitoring Committee
IEC:	Independent Ethics Committee
IgG:	Immunoglobulin G
IM:	Intramuscular
IMP:	Investigational Medicinal Product
IN:	Intranasal
IRB:	Institutional Review Board
LL:	Lower Limit
LPS:	Lipopolysaccharide
LSLV:	Last Subject Last Visit
MACDP:	Metropolitan Atlanta Congenital Defects Program
MedDRA:	Medical Dictionary for Regulatory Activities
MSD:	Moderate to Severe Diarrhea
OAg:	O antigen
PBMC:	Peripheral Blood Mononuclear Cells
PCD:	Primary Completion Date
PPS:	Per-Protocol Set
qPCR:	quantitative Polymerase Chain Reaction
S. sonnei:	*Shigella sonnei*

SAE:	Serious Adverse Event
SBA:	Serum Bactericidal Assay
SBIR:	Source DataBase for Internet Randomization
SDB:	Source Document Verification
sIgA:	Secretory Immunoglobulin A
SPM:	Study Procedures Manual
TMP-SMX:	Trimethoprim-sulfamethoxazole
TSA:	Trypticase soy agar
VE:	Vaccine Efficacy
WRAIR:	Walter Reed Army Institute of Research

GLOSSARY OF TERMS

Adequate contraception: Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- Abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- Combined estrogen and progesterone oral contraceptives,
- Injectable progestogen,
- implants of etonogestrel or levonorgestrel,
- Contraceptive vaginal ring,
- Percutaneous contraceptive patches,
- Intrauterine device or intrauterine system,
- Male partner sterilization prior to the female subject's entry into the study, and this male is the sole partner for that subject,

  The information on the male sterility can come from the site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- Male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository), and/or progesterone alone oral contraceptive.

Adequate contraception does not apply to subjects of childbearing potential with same sex partners, or for subjects who are and will continue to be abstinent from penile-vaginal intercourse on a long term and persistent basis, when this is their preferred and usual lifestyle.

Adverse event: Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Adverse Event of Special Interest:	Adverse events of special interest (AESIs): are predefined (serious or non-serious) adverse events of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate, because such an event might warrant further investigation in order to characterize and understand it.
Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In an observer-blind study, the subject and the site and sponsor personnel involved in the clinical evaluation of the subjects are blinded while other study personnel may be aware of the treatment assignment (see Section 6.2.3 for details on observer-blinded studies).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study: (Synonym of End of Trial)	For studies without collection of human biologicals samples or imaging data EoS is the last subject last visit (LSLV). For studies with collection of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after LSLV.
Epoch:	An epoch is a set of consecutive timepoints or a single timepoint from a single protocol. Epochs are defined to support a main purpose which is either to draw conclusions on subject participation or to draw a complete conclusion to define or precise the targeted label of the product. Supporting means that data collected at the timepoints included in an epoch must be sufficient to fulfil the purpose of the epoch. Typical examples of epochs are screening, primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
eTrack:	GSK's tracking tool for clinical trials.

Immunological correlate of protection:	The defined immune response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine: (Synonym of Investigational Medicinal Product)	A pharmaceutical form of an active ingredient being tested in a clinical trial, including a product with a marketing authorization when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses, and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Pharmacogenomics:	The International Council for Harmonisation (ICH) E15 Guidance for Industry defines pharmacogenomics as Study of variation of DNA and RNA characteristics as related to drug or treatment response. Pharmacogenetics, which is a subset of pharmacogenomics, is “the study of variations in DNA sequence as related to drug response.” Pharmacogenomic biomarkers include germline (host) DNA and RNA as well as somatic changes (e.g., mutations) that occur in cells or tissues. Pharmacogenomic biomarkers are not limited to human samples but include samples from viruses and infectious agents as well as animal samples. The term pharmacogenomic experiment includes both the generation of new genetic or genomic (DNA and/or RNA) data with subsequent analysis as well as the analysis of existing genetic or genomic data to understand drug or treatment response (pharmacokinetics, safety, efficacy or effectiveness, mode of action). Proteomic and metabolomic biomarker research are not pharmacogenomics.

Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the clinical trial was concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Council for Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Randomization:	Process of random attribution of treatment/schedule to subjects in order to reduce bias of selection.
Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Study vaccine/product:	Any investigational vaccine/product being tested and/or any authorized use of a vaccine/ product /placebo as a reference or administered concomitantly, in a clinical trial that evaluates the use of an investigational vaccine/product.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Subset: (Synonym of Immuno-subset)	Selection of blood samples among all blood sample collected at given timepoint(s) for testing by specific assay.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject.

Treatment number:	A unique number identifying a treatment to a subject, according to treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited adverse event.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines/products and/or medications will be written without the superscript symbol ™ or ® and in *italics*.

Trademarks not owned by the GSK group of companies	Generic description
Alhydrogel (Brenntag Nordic A/S [Brenntag Biosector A/S])	Aluminium Hydroxide, Hydrated, for Adsorption (Alhydrogel 2% - Ph.Eur.)

1. INTRODUCTION

1.1. Background

Shigellosis is a major public health problem around the world; it is one of the leading causes of diarrheal disease in low and middle income countries and is estimated to cause an all age mortality of 164 300 deaths every year [GBD, 2015]. The majority of these cases occur in children less than 5 years of age living in middle and low income countries. Currently, there are no vaccines licensed for use in these populations (except for China) and available interventions are under threat from the increasing rates of antibiotic resistance of *Shigella sonnei* (*S. sonnei*) around the world. Natural infection generates a serotype specific protective immune response against the O antigen (OAg) of the bacterial lipopolysaccharide (LPS). Previously, *S. sonnei* and *Shigella flexneri* type 2a were selected for a prototype OAg conjugate vaccine that produced a protective efficacy of 70% in children three years of age and older. However, there was no noted efficacy in children younger than three years of age, thus necessitating new approaches to vaccine development. Additionally, it is likely that a multivalent OAg vaccine will be required in order to achieve broad protection against the 4-6 serotypes causing the largest burden of disease in young children living in sub-Saharan Africa and South Asia.

Please refer to the current Investigator Brochure for information regarding the pre-clinical and clinical studies with the 1790GAHB vaccine and the epidemiological information.

1.2. Rationale for the study and study design

1.2.1. Rationale for the study

GlaxoSmithKline (GSK) Vaccines Institute for Global Health (GVGH) has developed a vaccine against *S. sonnei* (1790GAHB vaccine) using the new Generalized Modules for Membrane Antigens (GMMA) platform technology that in future could be also used to develop vaccines against other gram negative pathogens.

The study vaccine could be the stepping stone for the development of a multivalent broadly protective Shigella vaccine for vaccination of impoverished communities where shigellosis is endemic. However, a standalone monovalent vaccine against *S. sonnei* could be used to protect travelers against diarrheal shigellosis, as the vast majority of travelers' shigellosis is caused by *S. sonnei*, and even to protect infants in endemic regions where shigellosis is primarily caused by *S. sonnei*.

The 1790GAHB vaccine has been tested in two Phase I dose escalation studies in Europe to assess its safety and immunogenicity via three routes of administration: intramuscular (IM), intranasal (IN) and intradermal (ID). The results from the first study (dose escalation with IM vaccination) have shown that the vaccine has an acceptable safety profile and is well-tolerated up to a dose of 100 µg. Additionally, the serologic assessment showed that one dose of ≥ 1.5/25 µg OAg/protein of 1790GAHB vaccine elicits serum immunoglobulin G (IgG) anti-*S sonnei* LPS levels ≥ 121 enzyme-linked

immunosorbent assay (ELISA) units/mL (EU/mL) (i.e., median antibody titer in a panel of 87 Israeli convalescent subjects after natural infection by *S. sonnei*). The results from the second study (dose escalation with ID, IN and IM vaccination) showed that 1790GAHB vaccine is well-tolerated also when administered by the ID and IN routes of vaccination. However, immunogenicity data have shown that 1790GAHB vaccine administered by the ID and IN routes is not as immunogenic as 1790GAHB vaccine administered by the IM route. Therefore, it has been decided to proceed with the clinical development program of this vaccine only using the IM vaccination route. In terms of dosage, the regimen tested in Phase I studies (three doses given one month apart) did not show any significant benefit from the third dose in terms of immunogenicity, therefore a two dose schedule was selected for next studies.

A Phase IIa study, conducted in endemic regions of Africa (i.e., Kenya), has just been completed and confirmed the acceptable safety profile and immunogenicity of 1790GAHB vaccine.

Performing a vaccine-human challenge study may give the opportunity to establish evidence of clinical protection induced by the candidate *S. sonnei* vaccine (1790GAHB vaccine) at an early development stage.

1.2.2. Rationale for the study design

A human challenge model of *S. sonnei* disease for vaccine testing was established in Thailand [Bodhidatta, 2012] and showed an attack rate (AR) of 75% in non-immune adult volunteers receiving a challenge dose of 1680 colony forming units (CFU) of *S. sonnei* strain 53G. Clinical and laboratory endpoints of infection and disease, namely diarrhea, dysentery, fever and shedding of *S. sonnei* in stools were used to calculate the AR and thus to establish the optimal infective dose. In a subsequent study though [Pitisuttithum, 2016], the AR in the control group, using this model, was much less than previously published.

Given this variability, before starting a vaccine-challenge study, the proposed human challenge model of *S. sonnei* to be used has been appropriately developed and the challenge dose and AR reliably established by the Cincinnati Children's Hospital Medical Center (CCHMC) (Ohio, United States of America). The current GSK vaccine-human challenge study will be conducted at CCHMC and will use the developed challenge model. Volunteers will be treated after development of symptoms of shigellosis.

1.2.3. Rationale for the use of placebo

As currently there is no widely available vaccine against shigellosis, the efficacy and safety of the 1790GAHB vaccine will be evaluated against a placebo. The placebo is used to better describe the safety profile of the *S. sonnei* vaccine as well as its efficacy in terms of clinical protection against shigellosis caused by *S. sonnei*.

1.3. Benefit:Risk assessment

Please refer to the current Investigator Brochure for the summary of potential risks and benefits of the 1790GAHB vaccine.

The following section outlines the risk assessment and mitigation strategy for this study protocol:

1.3.1. Risk assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational vaccine: 1790GAHB vaccine		
Symptomatic Neutropenia	Eight cases of Grade 2 or Grade 3 neutropenia were reported from Phase 1 clinical trials (asymptomatic). All cases occurred in the first 20 days after administration of first vaccine dose, and reversed after discontinuation. Literature review of reports of neutropenia in association with vaccination indicates factors noted to be associated with neutropenia (including ethnicity, and low baseline counts) with the majority of cases detected within 2 weeks of the vaccination. None of the reported cases was clinically symptomatic. Five cases of asymptomatic neutropenia (Grade 1 and 2) have been reported at Day 7 after receipt of the first dose in 5 subjects. One of the 5 subjects had an additional Grade1 neutropenia episode at Day 28, and another one had two additional episodes, one of which was Grade 3 (940 cells/mL) at 7 days after the second dose and the second was Grade 2 (1,410 cells/mL) at 28 days after second vaccination. All cases were resolved before last study visit.	Exclusion criteria for this clinical study include: • Subjects with a low baseline neutrophil count below 1800 cells/µL (LLN). • Previous history of Benign Ethnic Neutropenia, or drug related Neutropenia. • Concomitant treatment with neutropenic agents. Monitoring for this clinical study to include: • Baseline neutrophil counts. • Full blood counts with differential measured at 7 days post each scheduled vaccination. • Assessment of clinical impact of neutropenia. Subjects will be closely monitored during the entire study and have hematology repeated every 7 days when neutropenia is identified until resolution. If a case of neutropenia does not resolve based on most recent available laboratory results, before the second vaccination, or challenge agent administration, the subject will not be re-vaccinated or administered the challenge agent.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Study Procedures		
Risks related to blood sample collection	When blood samples are taken from a vein, there is a risk of bruising at the site. There is a rare possibility of bleeding, fainting and infection or of nerve injury soreness.	Safe sampling procedures will be applied by appropriately trained and qualified staff to minimize distress and sampling errors. The potential risk of feeling faint, or experiencing mild local pain, bruising, irritation or redness at the site where blood was taken, is mentioned in the informed consent form. The amount of blood to be taken for sampling will not be harmful to the subject's health.
Risk related to rectal swabs	Rectal swabs may cause discomfort for patients and in case of special conditions (e.g. hemorrhoids, and rectal polyps) bleeding might happen.	Rectal swabs are only collected when it is not possible to obtain a stool sample. Study staff will instruct the subjects on how to do the rectal swab.
Other (challenge phase)		
Severe and invasive diarrhea after challenge	Illness caused by Shigella ranges from mild (watery diarrhea being the main symptom) to severe illness (fever and abdominal cramping with frequent small volume blood streaked stools containing mucus), dehydration and even shock. Perianal soreness, which is a skin irritation on your buttocks as a result of diarrhea, is possible.	Administration of an effective antibiotic will significantly reduce, or eliminate, symptoms within 24 hours of initiation. In addition, all challenged subjects will receive an effective antibiotic on the 5th day after challenge or earlier as required and intensive care unit is available to provide appropriate care in case of emergency. If perianal soreness occurs, it can be treated with topical lotion.
Risk of bacteremia after challenge	Bacteremia can occur with any invasive bacterial infection not appropriately contained with adequate antibacterial therapy.	The *S. sonnei* 53G challenge strain used in the challenge phase is known to be susceptible to multiple antimicrobial agents including Trimethoprim-Sulfamethoxazole (TMP-SMX) and quinolones which will be used in this study. To ensure there is no spread of shigella in the environment, subject will be discharged only following two negative Shigella cultures in stools.
Reiter's syndrome	A post-dysenteric reactive arthritis, with ocular and/or urethral inflammation, which has an onset typically 1 to 3 weeks after the onset of diarrhea, has been reported to occur in patients with shigellosis.	Data suggest that the risk of reactive arthritis following shigellosis may be higher in persons carrying the human leukocyte antigen (HLA)-B27, thus the exclusion of subjects with a positive HLA-B27 test at screening.

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Other (challenge phase)		
Risk related to antibacterial therapy	There are documented side effects with the use of antibiotics.	However, ciprofloxacin, the antibiotic given to all subjects on the 5th day after challenge, is generally well-tolerated. The most frequently reported drug related events for all indications of ciprofloxacin therapy have been nausea, diarrhea, abnormal liver function tests, vomiting, and rash which are all mild to moderate in most cases. The most common symptoms that happen after taking TMP-SMX are nausea, vomiting, loss of appetite, skin rash and itching. All subjects will be monitored closely and given adequate care when the need arises.

1.3.2. Benefit assessment

There may not be a direct medical benefit to the subject as a result of taking part in this study. However, the study vaccine may provide protection against *S. sonnei* bacteria as well as the administered challenge agent.

The subject, by participating in this study may help provide data about the tolerability of the experimental vaccine and about its ability to stimulate the immune system to produce antibodies against *S. sonnei* and provide clinical protection.

1.3.3. Overall benefit: Risk conclusion

Based on the clinical experience with 1790GAHB vaccine, tested in clinical studies, it is expected that 1790GAHB vaccine will also have an acceptable safety profile in future clinical studies and will be associated with clinically acceptable local and systemic reactions. In previous studies, the vaccine has shown good immunogenicity. If immunogenicity will correlate to clinical protection in this proposed study, the vaccine will help protect the subjects who received it from shigellosis caused by *S. sonnei*. However, a similar benefit is not anticipated for subjects who will receive placebo. Overall, all subjects may help in the characterization of the vaccines' immunogenicity and ability to provide protection. Administration of the challenge agent causes discomfort and illness that ranges from mild (watery diarrhea being the main symptom) to severe (fever and abdominal cramping with frequent small volume blood streaked stools containing mucus) diarrhea. However, the *S. sonnei* 53G challenge strain used in the challenge phase is known to be susceptible to multiple antimicrobial agents including TMP-SMX and quinolones which will be used in this study and will be given to all challenged subjects 5 days after administration of the challenge agent (day 62) or earlier if medically needed. Additionally, should any allergic reaction occur after challenge administration, an intensive care unit with medications is available at the study site in order to treat the possible anaphylactic/allergic reactions.

2. OBJECTIVES

2.1. Primary objective

- To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo (reduction of shigellosis according to the protocol primary case definition, i.e. Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea, OR shedding with an oral temperature of ≥ 38.5°C after challenge with *S. sonnei* strain 53G*)*.

  Criterion:

  Vaccine efficacy (VE) of 1790GAHB vaccine will be shown if the lower limit (LL) of the 90% confidence interval (CI) of VE will be above zero.

Refer to Section 4 for the protocol primary case definition of shigellosis and to Section 11.1 for the definition of the primary endpoint.

2.2. Secondary objectives

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - Shigellosis as defined by the CHIM expert working group on case definition.*
  - Shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].
  - More severe shigellosis, as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom]]. ***(Amended: 23 July 2019)***
  - Shedding of *S. sonnei* strain 53G.
  - Severe diarrhea.
  - More severe diarrhea.
  - Dysentery.
  - Weight of all grade 3-5 stools.
  - Total number of all grade 3-5 stools.
  - Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia).

- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- Time to onset of shigellosis after challenge, according to the primary case definition.

*According to the working group, the participant must fulfil any one of the three following possible endpoints to qualify as having reached the CHIM case definition for this objective:

1. Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]
2. Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]
3. Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].

Safety

- To assess the safety and reactogenicity of the 1790GAHB vaccine in terms of solicited adverse events, unsolicited adverse events, serious adverse events (SAEs), adverse events of special interest (AESI) and laboratory parameters.
- To assess safety after challenge in terms of SAEs, unsolicited adverse events, AESI, and laboratory parameters.

Immunogenicity

- To evaluate the IgG ELISA immunogenicity profile of the 1790GAHB vaccine at 7 days and 28 days after the first and second vaccination (IgG ELISA coated with OAg containing LPS).
- To evaluate the immunogenicity profile of the 1790GAHB vaccine at pre-challenge and at 7 and 28 days after challenge (IgG ELISA coated with OAg containing LPS).
- Number and percentage of seroresponders for anti-S. sonnei LPS at 28 days after first and second vaccination.
- To assess anti- S.sonnei LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.

Refer to Section 11.2 for the definition of the secondary endpoints.

2.3. Tertiary objectives

The following tertiary objectives which are part of the study exploratory objectives may be evaluated in addition to the primary and secondary objectives and will complement assessment of the vaccine immunogenicity profile:

- Evaluate *S. sonnei* specific secretory immunoglobulin A ***(***sIgA***)*** in stool at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate ***S. Sonnei LPS IgG-specific*** α4β7***+/-*** plasmablast ***response*** at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64). ***(Amended: 23 July 2019)***
- Evaluate serum bactericidal activity against *S. sonnei* at Day 1 and 28 days after first and second vaccination.
- Evaluate correlation between serum anti-*S. sonnei* LPS IgG level, serum bactericidal activity titer, shigellosis, shedding, mild, moderate, severe or more severe diarrhea, and dysentery after challenge.
- Evaluate glycosylation of fragment crystallisable (Fc) portion of IgG antibodies at Day 1 and 28 days after second vaccination and association with clinical protection and/or serological endpoints.
- Evaluate inflammatory response from stools collected before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64).
- Evaluate gene expression signatures at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Investigate gut microbiome at Day 1 and 28 days after second vaccination and its potential association with protection and immune response.

  These last two tertiary objectives involve pharmacogenomics testing. Refer to the glossary of terms for the definition of Pharmacogenomics.

Refer to Section 11.3 for the definition of the tertiary endpoints and to Section 11.12.1 for the reporting of tertiary endpoint results.

3. STUDY DESIGN OVERVIEW

This study will evaluate the efficacy, safety and immunogenicity of the 1790GAHB vaccine administered via IM route to adults (18-50 years of age at enrolment). The objectives will be assessed through the conduct of the study in two phases: a vaccination phase with 1790GAHB vaccine or placebo followed by a challenge phase with *S. sonnei* strain 53G.

The total of 72 subjects will be divided in 4 cohorts of 18 subjects each (for logistical reasons due to bed capacity at the study site). The vaccination and challenge will be done in overlapping cohorts and randomization will ensure there is the same number of vaccines and controls in each cohort. However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.

3.1. Vaccination Phase

During the screening period before the first vaccination (Day -45 to Day -1), subjects providing informed consent will have their medical history recorded and be screened for general health status.

Female subjects with childbearing potential must use acceptable birth control measures (defined as hormonal [e.g., oral, injection, transdermal patch, implant, cervical ring], barrier [e.g., condom with spermicide or diaphragm with spermicide], intrauterine device, or be in a monogamous relationship with a partner who has been vasectomized for 6 months or more prior to the subject's study entry) during the entire study participation. Refer to the glossary of terms for the definition of adequate contraception.

At the end of the screening, subjects will be evaluated for eligibility. Only subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for enrolment. Following informed consent and confirmation of eligibility according to the inclusion and exclusion criteria, subjects will be randomized in a 1:1 ratio to receive either the study vaccine or the placebo. Subjects will receive two vaccinations, 28 days apart.

Subjects will be observed at the site for at least 30 minutes after each vaccination. All subjects will have their vital signs (temperature, heart rate, sitting blood pressure) taken before vaccination and at the end of the observation period to ensure they are stable before they go home. Subjects will be provided with a diary card and will be trained on how to fill it in. Subjects will be reminded before they go home of the importance of filling the diary card and a phone call will be performed by the site staff to the subjects at 2 and 6 days following each vaccination to remind them that the diary card should be completed (no update on the status of the subject's health will be solicited during these phone calls, as they are not intended for safety data collection) and of the clinical visit at 7 days after each vaccination. The diary card will be used to collect all solicited and unsolicited AEs, and medications for the first 7 days after each vaccination starting 6 hours after the administration of the vaccine/placebo. At 7 days following each vaccination and until 28 days after vaccination, only unsolicited AEs, solicited adverse reactions that continue at the 7th day, and related medications will be collected during visits at the study site. Any AE reported in the diary card beyond the 7th day will be followed up accordingly and the frequency of the follow-up will depend on its nature.

Prior to each vaccination, subjects will be assessed through their medical history since the last visit and physical examination to check if continued participation in the study is in their best interest and that there are no contraindications for vaccination (e.g. fever or acute illness on the day of vaccination). Subjects will also be evaluated for continued eligibility with regard to the inclusion and exclusion criteria. Vaccination will only take place after the subjects' medical history, clinical examination and evaluation of inclusion and exclusion criteria confirm his/her continuous eligibility. Investigator judgment will also apply to evaluate subjects' continued eligibility in the study. Subjects will be encouraged to visit the study clinic or call the study staff if they have fever or are unwell between planned visits. This, depending on the nature, may prompt an unscheduled visit by a study physician. Any Grade 3 or more reaction after vaccination may also prompt a review by a study physician at the study clinic.

3.2. Challenge Phase

Four weeks after completion of the two-dose vaccination schedule (approximately 28 days after Visit 3) subjects will be admitted the day before the challenge date (Day 56) and will stay for 9 days in the inpatient unit. Biological sample collection could start for logistical reason at Day 56 (the day of admission before challenge). The day of challenge (Visit 5; Day 57), they will be administered a 1500 CFU challenge dose of *S. sonnei* 53G. Before receiving the challenge, all inclusion and exclusion criteria and hematology will be assessed.

When assessing inclusion and exclusion criteria, the investigator will also inquire about antibiotic treatment during the week prior to the challenge date. Any subject who had a course of antibiotic the week prior to challenge, will not receive the challenge agent.

Subjects will fast for 90 minutes prior to receiving the *S. sonnei* 53G challenge inoculum and an additional 90 minutes after challenge. All subjects in the same cohort will be challenged in the same day using the same inoculum, which will ensure the consistency of the dose used. Approximately 5 minutes before drinking the challenge suspension, the subject will drink a 120 mL solution of sodium bicarbonate to neutralize gastric acidity. A subject with fever or signs of acute illness will not receive the challenge agent and any subject who vomits following ingestion of the challenge agent will not be re-dosed. A note will be made in the subject source documents regarding the apparent amount of the vomitus (small, moderate or large). The subject will still continue in the study and undergo all study-related procedures. Care will be taken to ensure that a minimum amount of time, not to exceed 2 hours, is spent between the reconstitution of the challenge inoculum and the oral administration of the inoculum to the subject.

Two mL of the inoculum will be retained for pre- and post-challenge quantification of the dose. Aliquots of the retained inoculum will be streaked onto trypticase soy agar (TSA) plates and after incubation, average number of colonies on the TSA plates will be counted and CFU/mL will be calculated. Study staff will take all necessary precaution needed for handling of *S. sonnei* 53G and adhere to the CCHMC policy and procedures.

During the first 8 days in the inpatient unit (Day 57 to Day 64) following challenge, subjects will be monitored daily for AEs, gastrointestinal or other systemic signs and symptoms. Subjects will be assessed for vital signs (including sitting blood pressure, temperature and pulse rate) according to local standards for inpatient care. Stool analysis will be carried out daily during the inpatient stay. All stools will be collected and visually assessed for consistency, blood or mucus. Stools that are loose or watery (grades 3-5) will be weighed, and if there is visible blood, a Hemoccult test will be performed to confirm presence of occult blood. Up to two stools with blood present by visual inspection in a 24 hour period will be tested by Hemoccult. Study staff will record the information in the subject's source document. If a subject is unable to produce a stool by midnight, a rectal swab will be obtained (up to 2 adequately collected swabs per day). Study staff will record the information in the subject's source document. The first stool sample collected during a 24 hour period on the day after challenge agent administration (from 00:01 to 24:00) will be analyzed by culture and quantitative polymerase chain reaction (qPCR) specific for *S. sonnei* 53G. The day before discharge (Day 64), two stool samples will be collected to confirm that the subject is no longer shedding. Instructions for appropriate

collection, transportation and storage of stool samples will be provided to the investigators. For diarrhea episodes to be considered as separate episodes, there should be at least a 6-hour interval between them (refer to Section 4 for details on stool grading).

On the 5th day after challenge (Day 62), subjects will receive as first-line treatment 500 mg of ciprofloxacin twice daily for 3 days. Subjects with contraindication to ciprofloxacin may receive as second-line treatment trimethoprim-sulfamethoxazole (160 mg/800 mg twice daily), amoxicillin (500 mg three times daily) for 5 days, or any antibiotic that is deemed suitable according to local guidelines and the investigator's assessment can be used.

Subjects may be treated with antibiotics before the 5th day after challenge (Day 62) if:

- They have diarrhea (any severity stool Grade 3 or higher) **and** two or more of the following symptoms: severe abdominal pain/cramps, severe nausea, severe headache, severe myalgia, severe arthralgia, gross blood in ≥ 2 stools, any fever (≥ 38.0ºC measured orally), or any vomiting.
- They experience unexpectedly severe events such as hypotension (disproportionate to volume loss), renal dysfunction, or altered mental state (e.g. somnolence) at the discretion of the investigators.
- A study physician determines early treatment is warranted for other reasons.

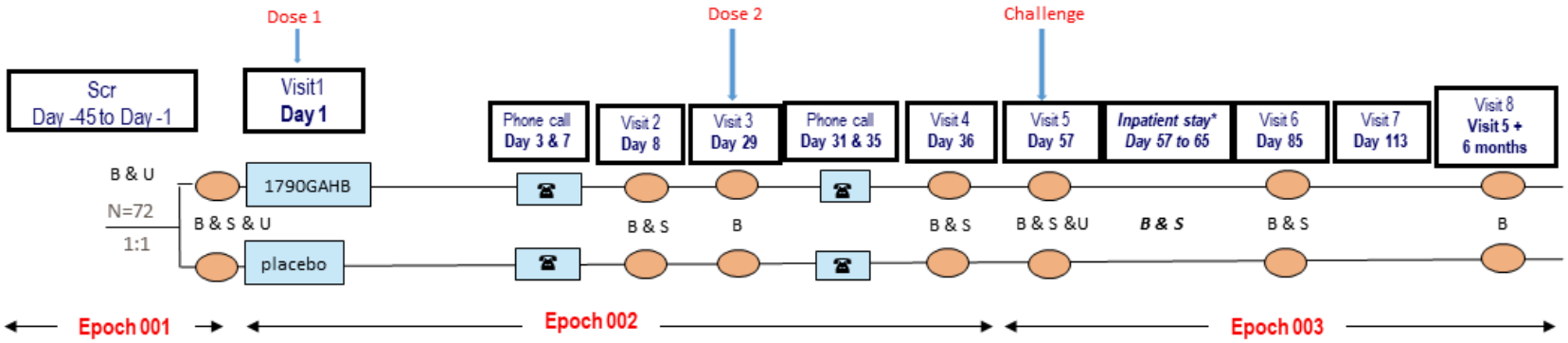
A subject receiving early antibiotic treatment will still remain on the unit until the planned day of discharge (the 9th day of inpatient stay: Day 65). On that day, if a subject is clinically well and has had at least 2 negative stool cultures, collected at least 6 hours apart, for *S. sonnei*, s/he will be discharged. A subject that, on the planned day of discharge, still had S. *sonnei* isolated from the Day 64 stools will be asked to remain in the unit until s/he is no longer shedding. A subject still shedding 48 hours after initiation of ciprofloxacin will be switched to a Septra DS (1 tablet twice a day for five days). If a subject is still positive 48 hours after the second-line antibiotic is administered, a culture and antibiotic sensitivity test will be obtained and therapy initiated based on sensitivity results. If a subject has two consecutive negative stool samples and is eligible to be discharged from the inpatient stay but did not finish his treatment course, s/he can be given the remaining doses to complete at home.

Following discharge, subjects will return at 28 days (Visit 6), 56 days (Visit 7) and 6 months (Visit 8) after challenge for outpatient clinical assessment. During these visits, clinical assessment will be done to assess their general health status and find out about the occurrence of AESI, any AE and SAE.

Subjects will be advised at the time of their discharge to contact the study staff/doctor if they feel unwell. The subjects will be followed up until 6 months after the challenge. At the last study visit, at 6 months after challenge, the subject will be reviewed for the occurrence of any AESI and the site staff will ensure all AEs are resolved.

Through the entire study, all subjects will be advised to report to the study facility whenever they are sick. Standard patient care, according to local guidelines will be provided for all illnesses in both the outpatient and inpatient facilities. Additional investigations may be conducted if the investigator considers them necessary to better evaluate the subject's condition. An overview of the study design is given in Figure 1.

Figure 1 Study design (Amended: 23 July 2019)



Refer to Section 3.2 for details of procedures during the inpatient stay (Amended: 23 July 2019)
Scr: Screening
N: Number of subjects
B: Blood sampling
S: Stool sampling
U: Urine sampling

Protocol waivers or exemptions are not allowed unless necessary for the management of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6.4.1), are essential and required for study conduct.

- **Experimental design:** Phase IIB, observer-blind, randomized, placebo control, mono-centric study with two parallel groups (*S. sonnei* group and placebo group).
- **Duration of the study**: Each subject will be followed up for approximately 6 months after challenge with the pathogenic *S. sonnei* strain 53G, with a total study duration of approximately 32 weeks (i.e., 8 months) for each study subject.
  - Epoch 001: Starting at Screening Visit (Day -45 to Day -1) and ending before Visit 1 (Day 1).
  - Epoch 002: Starting the day of randomization and first vaccination (Visit 1) and ending before the receipt of the challenge agent, 28 days after second vaccination (Visit 5).
  - Epoch 003: Starting with the receipt of the challenge agent at 28 days after second vaccination (Visit 5) and ending 6 months after challenge (Visit 8).
- **Primary completion date (PCD):** ***End of inpatient stay (Visit 5, Day 64)***. ***(Amended: 23 July 2019)***

  Refer to glossary of terms for the definition of PCD.
- **End of Study (EoS):** Last testing results released of samples collected for primary and secondary objectives at Visit 8.

  Refer to glossary of terms for the definition of EoS.
- **Study groups:**

Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min - Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
			Screening	1st and 2nd vaccination	Challenge
S. sonnei	36	18 years – 50 years	x	x	x
Placebo	36	18 years – 50 years	x	x	x

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/ placebo/ challenge agent name	Study Groups	
		S. sonnei	Placebo
S. sonnei	1790GAHB	x	
Placebo	GAHB-Placebo		x
Challenge	*S. sonnei* 53G strain	x	x

- **Control:** Placebo control with GAHB-Placebo with the same composition as the vaccine except the active ingredient. GAHB-Placebo will also be used as diluent for bedside mixing of the vaccine.

- **Vaccination schedule**: Subjects will receive 2 doses of either the study vaccine or placebo 28 days apart. At 28 days after the second dose, all subjects will receive the challenge dose.
- **Treatment allocation**: Following the screening period before the first vaccination (Day -45 to Day -1), subjects will be randomized in a 1:1 ratio to receive either the study vaccine or the placebo.
- **Blinding**: The study will be observer-blind.

Table 3 Blinding of study epochs

Study Epochs	Blinding
Screening (Epoch 001)	N/A
1st and 2nd vaccination (Epoch 002)	observer-blind
Challenge (Epoch 003)	observer-blind

- **Sampling schedule:**
  - 5 mL for hematology: Screening Visit, Visit 2, Visit 3, Visit 4, Visit 5 and Visit 8. Additionally, hematology is repeated weekly until resolution if neutropenia occurs.
  - 5 mL for biochemical analysis: Screening Visit.
  - 10 mL for hepatitis B, hepatitis C and HIV: Screening Visit.
  - 10 mL for HLA-B27 testing: Screening Visit.
  - 5 mL for local anti *S. sonnei* LPS IgG Screening-ELISA: Screening Visit.
  - Urine sample for urinalysis: Screening Visit and Visit 5.
  - Urine sample for pregnancy test for women of childbearing potential: Screening Visit, Visit 1, Visit 3, Visit 5 and Visit 8.
  - 20 mL for immunological tests (antibody response) at Visit 1, Visit 2, Visit 3, Visit 4, Visit 5, 7 days after Visit 5 (Day 64), Visit 6,
  - For serum bactericidal activity tests at Visit 1, Visit 3 and Visit 5
  - And for Fc glycosylation test on leftover serum samples collected at Visit 1 and Visit 5.
  - 50 mL of peripheral venous blood for α4β7+/- plasmablast ***analysis by ELISA*** and RNA extraction from PBMCs for gene expression profiling: Visit 1, Visit 2, Visit 4 and 7 days after Visit 5 (Day 64). ***(Amended: 23 July 2019)***
  - Stool sample for inflammatory response before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64)**.**
  - Stool sample: for microbiome testing at Visit 1 and Visit 5, for *S. sonnei* specific sIgA at Visit 1, Visit 2, Visit 4 and 7 days after challenge (Day 64) and stool analysis, culture and qPCR daily during inpatient stay (from Visit 5 to 7 days after challenge [Day 57 to Day 64] or prolonged until 2 consecutive negative stool samples if subject still shedding).

- **Type of study**: self-contained.
- **Data collection**: Standardized electronic Case Report Form (eCRF).
- **Safety monitoring:** An Independent Data Monitoring Committee (IDMC) will be established to monitor the safety of subjects throughout the study. The IDMC will review unblinded data, to assess safety signals and make recommendations to the Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects. The membership and roles of the IDMC will be outlined in the IDMC charter.

  The safety review team (SRT) will monitor the safety of subjects throughout the study. The SRT will review blinded data, assess safety signals and make recommendations to the IDMC and Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects.

  A dedicated Safety Management Plan will be prepared to ensure appropriate safety reporting responsibilities and roles are defined.

  Refer to Section 9.10 for detailed description of holding rule and safety monitoring.

4. CASE DEFINITIONS

Stools will be graded as follows:

- Grade 1: firm formed;
- Grade 2: soft formed;
- Grade 3: viscous opaque liquid or semi-liquid which assumes the shape of the bowl;
- Grade 4: watery opaque liquid;
- Grade 5: clear watery or mucoid liquid.

4.1. Diarrhea

For the purpose of this study, diarrhea is defined as:

- Moderate diarrhea: 4 to 5 loose or watery (Grade 3 to 5) stools or 400 to 800 grams of Grade 3 to 5 stools within 24 hours.
- Severe diarrhea: 6 or more loose or watery (Grade 3 to 5) stools or > 800 grams of Grades 3 to 5 stools within 24 hours or required medical intervention. In case of severe diarrhea, medical intervention is defined as intravenous (IV) fluids administration or anticipation of antibiotic treatment before the 5th day after challenge.
- More severe diarrhea: 10 or more loose or watery (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours.

4.2. Dysentery *(Amended: 23 July 2019)*

For the purpose of the endpoint "Rate of shigellosis as defined by the CHIM expert working group occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]. In this case constitutional/enteric symptom are the following: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise.***

For the purpose of the endpoint "Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [≥1 reportable constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoint "Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]].", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoints "Dysentery" and "Confirmed S. sonnei 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia)", dysentery is defined as follows:

- ***A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours and presence of constitutional/enteric symptoms. In this case, constitutional/enteric symptoms consist of headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting, and fever.***

(Amended: 23 July 2019)

4.3. Shigellosis

For the primary and secondary objectives in the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea OR shedding with an oral temperature of ≥ 38.5°C).

For the secondary objectives in the challenge phase of the study, 2 definitions of shigellosis are used:

1. CHIM working group case definition for shigellosis as defined by any one of the three following possible endpoints:
   - Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]
   - Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]
   - Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].
2. Shigellosis as defined by any one of the three following possible endpoints:
   - Severe diarrhea defined as [≥6 loose stools in 24 hours OR >800 grams loose stools in 24 hours]
   - Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 moderate constitutional/enteric symptom].
   - Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom].

For the secondary objectives in the challenge phase of the study, more severe shigellosis is defined by any one of the two following possible endpoints:

- Moderate OR severe diarrhea [≥4 loose stools in 24 hours OR ≥400 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 severe constitutional/enteric symptom].
- Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0ºC OR*** ≥1 severe constitutional/enteric symptom]. ***(Amended: 23 July 2019)***

5. STUDY COHORT

5.1. Number of subjects/center

Target enrolment is up to 72 eligible subjects (36 per treatment group). Refer to Sections 5.2 and 5.3 for eligibility criteria and to Section 11.4 for the determination of sample size.

Enrolment will take place in 4 overlapping cohorts of 18 subjects each to accommodate for the availability of beds required for the challenge phase of the study at the clinical site (see also Section 11.12.1). However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort. The vaccination and challenge will be done per cohort.

5.2. Inclusion criteria

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- Written informed consent obtained from the subject prior to performing any study specific procedure.
- Individuals who, after the nature of the study has been explained to them, have shown adequate comprehension of the study procedures and knowledge of study.
- A male or female between, and including, 18 and 50 years of age at the time of the first vaccination.
- Healthy subjects as established by medical history and clinical examination before entering into the study.
- Seronegative for HIV, hepatitis B and C.
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause.

  Please refer to the glossary of terms for the definition of menarche and menopause.
- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - Has practiced adequate contraception for 30 days prior to vaccination, and
  - Has a negative pregnancy test on the day of vaccination, and
  - Has agreed to continue adequate contraception during the entire study period.

  Please refer to the glossary of terms for the definition of adequate contraception.

5.3. Exclusion criteria

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity, regulatory acceptability of the study or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry and at each vaccination and challenge administration visit. If ANY exclusion criterion applies, the subject must not be included in the study:

- Received an investigational or non-registered medicinal product within 30 days (Day -29 to Day 1) prior to informed consent, or planned use during the study period.
- Any behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the study.
- History of any neurological disorders or seizures.
- Any other clinical condition that, in the opinion of the investigator, might pose additional risk to the subject due to participation in the study.
- HLA-B27 positive test at screening and/or with history of reactive arthritis.
- Clinical conditions representing a contraindication to intramuscular vaccination and blood draws.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting six months prior to the first vaccine/placebo dose. For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent. Inhaled except for doses > 800 μg/day and topical steroids are allowed.
- Administration of long-acting immune-modifying drugs at any time during the study period (e.g. infliximab). Subjects may be on chronic or as needed medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition.
- Known bleeding diathesis or any condition that may be associated with a prolonged bleeding time.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational vaccine/product (pharmaceutical product or device).
- History of having participated in a previous Shigella challenge study.
- Individuals who have a previously laboratory confirmed case of disease caused by *S. sonnei* or serology positive for local anti *S. sonnei* LPS IgG Screening-ELISA at screening.
- History of any serious chronic or progressive disease according to judgment of the investigator (e.g., neoplasm, insulin dependent diabetes, cardiac, renal or hepatic disease).

- History of any malignancy or lymphoproliferative disorder.
- Known to be part of study personnel or being a close family member to the personnel conducting this study.
- History of anaphylactic reaction or allergy to vaccine/placebo or challenge agent components or any other allergies deemed by the investigator to increase the risk of an AE if they were to participate in the study.
- Known allergy to ciprofloxacin or the other antibiotics used for treatment as deemed by the investigator.
- Individuals receiving a course of antibiotics within a week of the challenge will be ineligible to receive the challenge strain.
- History of gastric acid hyper-secretory disorders (e.g., Zollinger-Ellison syndrome) as assessed and judged by the investigator or any other significant intestinal disorder.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Family history of congenital or hereditary immunodeficiency.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccine/placebo or challenge agent.
- Acute disease and/or fever at the time of enrolment.
  - Fever is defined as temperature ≥ 38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever may, be enrolled at the discretion of the investigator.

  Note: enrolment may be postponed/delayed until such transient circumstances have terminated.
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality, as determined by physical examination or laboratory screening tests. Chronic medical diagnoses or conditions should be stable for the last 60 days. This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrolment.
- A clinically significant sign or symptoms of acute illness, significant anomalies in vital signs.
- Known to handle food as part of work related activities.
- Hepatomegaly, right upper quadrant abdominal pain or tenderness.
- Received immunoglobulins or any blood products within 180 days prior to informed consent or planned administration during the study period.
- Pregnant or lactating female.

- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- Females with history of stillbirth, neonatal loss, or previous infant with anomaly, except those who have had a planned termination of pregnancy, hysterectomy or bilateral tubal ligation.
- History of chronic alcohol consumption and/or drug abuse. Chronic alcohol consumption is defined as: a prolonged period of frequent, heavy alcohol use; the inability to control drinking once it has begun; physical dependence manifested by withdrawal symptoms when the individual stops using alcohol; tolerance, or the need to use more and more alcohol to achieve the same effects; a variety of social and/or legal problems arising from alcohol use.
- Known to have close household or professional contacts with people with immunosuppressive condition.
- Documented HIV, hepatitis B and C positive subject.
- Any condition which, in the opinion of the investigator, may pose an increased and unreasonable safety risk to the subject if they participated in the study.
- Subjects with a baseline neutrophil count below 1800 cells/µL (LLN) OR with clinically significant abnormalities in other laboratory values (CBC, CMP, UA), according to local reference ranges and investigator judgment.
- Previous history of Benign Ethnic Neutropenia, or drug related Neutropenia.
- Concomitant treatment with neutropenic agents.

6. CONDUCT OF THE STUDY

6.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for GCP, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favorable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favorable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written informed consent must be obtained from each subject prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgment, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

6.2. Subject identification and randomization

6.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects who have consented to participate in the study, according to the range of subject identification numbers allocated to the study center.

6.2.2. Randomization of treatment

6.2.2.1. Randomization of supplies

The randomization of supplies for the Vaccination Phase (1790GAHB vaccine and placebo) within blocks will be performed by the study biostatistician or delegate. Entire blocks will be shipped to the study center. No randomization will be done for the challenge phase.

6.2.2.2. Treatment allocation to the subject

The treatment numbers will be allocated by dose. Throughout the study, a single treatment number will identify each dose to be administered to each randomized subject.

6.2.2.2.1. Study group and treatment number allocation

The target will be to enroll approximately 72 eligible subjects who will be randomly assigned to two study groups in a 1:1 ratio (approximately 36 subjects in each group).

Subjects will be enrolled in 4 overlapping cohorts of 18 subjects each, for logistical reasons at the clinical site, therefore a 1:1 ratio should be maintained at the cohort level. However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.

Allocation of the subject to a study group at the investigator site will be performed using the Source DataBase for Internet Randomization system (SBIR). The randomization algorithm might use a minimization procedure; more details are provided in the Statistical Analysis Plan.

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine/placebo administration will access SBIR. Upon providing the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for each dose.

A randomization blocking scheme (1:1 ratio) will be used to ensure the balance between treatments is maintained, accounting also for the enrolment cohorts: same number of vaccine and control recipients in each cohort.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual (SPM) for specific instructions.

6.2.3. Allocation of subjects to assay subsets

Leftover serum samples collected at Day 1 and 28 days after second vaccination for the antibody response analysis will be used for the Fc glycosylation test (immunological tests mentioned in the tertiary objectives) that will be performed at Rockefeller University (see Appendix B).

6.3. Method of blinding

Data will be collected in an observer-blind manner. By observer-blind, it is meant that during the course of the study, the vaccine/placebo recipient and those responsible for the evaluation of any study endpoint (e.g. safety, reactogenicity, immunogenicity and efficacy) will be unaware of which vaccine (study vaccine or placebo) was administered. To do so, vaccine/placebo preparation and administration will be done by authorized medical personnel who will not participate in any of the study clinical evaluation assays.

The laboratories in charge of the laboratory testing will be blinded to the treatment, subject and visit number, and codes will be used to link the subject, visit and study (without any link to the treatment attributed to the subject) to each sample.

6.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

6.4.1. Independent data monitoring committee

An IDMC will be established by GSK Biologicals for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The IDMC will provide recommendations about stopping, continuing or modifying the study. Refer to Section 9.10 for a description of the holding rule and safety monitoring put in place for this study.

6.5. Outline of study procedures

Table 4 List of study procedures

Epoch	Screening	Vaccination						Challenge				
Type of contact	Screening visit	Visit 1	Phone Call	Visit 2	Visit 3	Phone call	Visit 4	Visit 5	Inpatient stay	Visit 6	Visit 7	Visit 8
Timepoints (days)	Day -45 to Day -1	Day 1	Day 3 and Day 7	Day 8	Day 29	Day 31 and Day 35	Day 36	Day 57	Day 57 to Day 65 [8]	Day 85	Day 113	Day 237 (Month 8)
Sampling timepoints	Screening	Pre-vacc 1		Post-vacc 1	Pre-vacc 2		Post-vacc 2	Pre-challenge[9]	Inpatient stay	Post-challenge		
Informed consent	●											
Check inclusion/exclusion criteria	●				●			●				
Collect demographic data	●											
Measure/record height and weight	●											
Medical history	●											
History directed physical examination				O			O		O	O	O	O
Physical examination	●	●			●			●				
Urine pregnancy test [1]	●	●			●			●				●
Check contraindications to vaccination	O	O			O							
Pre-vaccination/placebo/challenge agent administration body temperature		●			●			●				
Vaccine/placebo/challenge agent administration												
Study group and treatment number allocation		●										
Recording of administered treatment number		●			●							
Vaccine/placebo administration		●			●							
Challenge with *S. sonnei* 53G								●				
Laboratory assays												
Blood sampling for antibody response (~20 mL)		● [2, 3]		●	● [2]		●	● [2, 3]	● [4]	●		

Epoch	Screening	Vaccination						Challenge				
Type of contact	**Screening visit**	**Visit 1**	**Phone Call**	**Visit 2**	**Visit 3**	**Phone call**	**Visit 4**	**Visit 5**	**Inpatient stay**	**Visit 6**	**Visit 7**	**Visit 8**
Timepoints (days)	**Day -45 to Day -1**	**Day 1**	**Day 3 and Day 7**	**Day 8**	**Day 29**	**Day 31 and Day 35**	**Day 36**	**Day 57**	**Day 57 to Day 65 [8]**	**Day 85**	**Day 113**	**Day 237 (Month 8)**
Sampling timepoints	**Screening**	**Pre-vacc 1**		**Post-vacc 1**	**Pre-vacc 2**		**Post-vacc 2**	**Pre-challenge[9]**	**Inpatient stay**	**Post-challenge**		
Blood sampling for PBMC isolation (α4β7**+/-** plasmablasts response and transcriptomics) (~50 mL) ***(Amended: 23 July 2019)***		●		●			●		● [4]			
Blood sampling for hematology (5 mL) [5]	●			●	●		●	●				●
Blood sampling for biochemical analysis (~5 mL)	●											
Serology for virology: HIV, HBV, HCV (~10 mL)	●											
Blood test for HLA-B27 and local anti *S. sonnei* LPS IgG (Screening-ELISA) (~15 mL)	●											
Urine sampling for urinalysis [6]	●							●				
Stool sample for sIgA		●		●			●		● [4]			
Stool sample for microbiome testing[7]		●						●				
Stool sample for inflammatory response								●	●			
Stool assessment: weight, consistency, blood and *S. sonnei* by culture and qPCR									●			
Safety assessment												
Record any concomitant medications/vaccinations	●	●		●	●		●	●	●	●	●	●
Distribution of diary cards		O			O							
Return of diary cards				●			●					
Diary card transcription by investigator				●			●					
Recording of solicited adverse events within 7 days post-vaccination				●			●					
Recording of unsolicited adverse events within 28 days post-vaccination				●	●		●	●				

Epoch	Screening	Vaccination						Challenge				
Type of contact	**Screening visit**	**Visit 1**	**Phone Call**	**Visit 2**	**Visit 3**	**Phone call**	**Visit 4**	**Visit 5**	**Inpatient stay**	**Visit 6**	**Visit 7**	**Visit 8**
Timepoints (days)	**Day -45 to Day -1**	**Day 1**	**Day 3 and Day 7**	**Day 8**	**Day 29**	**Day 31 and Day 35**	**Day 36**	**Day 57**	**Day 57 to Day 65 [8]**	**Day 85**	**Day 113**	**Day 237 (Month 8)**
Sampling timepoints	**Screening**	**Pre-vacc 1**		**Post-vacc 1**	**Pre-vacc 2**		**Post-vacc 2**	**Pre-challenge[9]**	**Inpatient stay**	**Post-challenge**		
Recording of solicited adverse events within 7 days post-challenge									●			
Recording of unsolicited adverse events within 28 days post-challenge									●	●		
Recording of AESI, SAEs and pregnancies	●	●		●	●		●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●		●	●		●	●	●	●	●	●
Study Conclusion												●

V: Visit, **D**: Day, **Vacc**: Vaccination; **HIV**: Human Immunodeficiency Virus, **HCV**: Hepatitis C Virus, **HBV**: Hepatitis B Virus, **ELISA**: Enzyme-linked immunosorbent assay, **AESI**: Adverse Events of Specific Interest, **SAEs**: Serious Adverse Events.

● is used to indicate a study procedure that requires documentation in the individual eCRF.

O is used to indicate a study procedure that does not require documentation in the individual eCRF.

[1] Female subjects will be randomized and study vaccine/placebo or challenge agent may only be administered if the pregnancy test is negative.

[2] Serum bactericidal assay will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response.

[3] Fc glycosylation test on leftover serum samples will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response and serum bactericidal assay.

[4] Sampling to be done 7 days after challenge agent administration (i.e., Day 64).

[5] Additionally, hematology is repeated weekly until resolution if neutropenia occurs. On the day of vaccination/challenge, sampling will be done before vaccination/challenge.

[6] Urine dipstick to be done for all subjects and, in case of abnormal results, a urine culture is to be performed (urinalysis).

[7] Before vaccination/challenge (Day 1/Day 57 sample can be collected by the subject at most a week before and stored as described in the Investigator Manual and SPM.

[8] Sampling to be done daily during inpatient stay and if subject is still shedding 7 days post-challenge administration (Day 64). However, all subjects even if not shedding anymore will remain in the inpatient stay until 7 days post-challenge and will be discharged on Day 65.

[9] Samples collection can be performed between Day 56 and Day 57.

Whenever possible, the investigator should arrange study visits within the interval described in Table 5.

Table 5 Intervals between study visits *(Amended: 23 July 2019)*

Interval	Optimal length of interval	Allowed interval
Screening visit → Visit 1	-7 days	-45 days – -1 days
Visit 1 → Phone Call	3 days	2 days - 6 days
Visit 1 → Visit 2	7 days	7 days - 10 days
Visit 1 → Visit 3	28 days	26 days - 33 days
Visit 3 → Phone call	3 days	2 days - 6 days
Visit 3 → Visit 4	7 days	7 days - 10 days
Visit 3 → Visit 5	28 days	26 days - 33 days
Visit 5 → Inpatient stay	9 days	9 days - as needed
Visit 5 → Visit 5 post-challenge blood draw (Amended: 23 July 2019)	***7 days (Amended: 23 July 2019)***	***7 days – 10 days (Amended: 23 July 2019)***
Visit 5 → Visit 6	28 days	28 days - 35 days
Visit 5 → Visit 7	56 days	49 days - 63 days
Visit 5 → Visit 8	180 days	166 days - 194 days

6.6. Detailed description of study procedures

6.6.1. Informed consent

The signed informed consent of the subject must be obtained before study participation.

Refer to Section 6.1 for the requirements on how to obtain informed consent.

6.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 5.2 and 5.3 before enrolment.

Subjects who meet all inclusion criteria and none of the exclusion criteria, with screening tests within normal values and women of childbearing potential with negative urine pregnancy test at Screening will be eligible for enrolment.

Continued participation of the subject in the study will also be checked against inclusion and exclusion criteria.

6.6.3. Collect demographic data

Record demographic data such as year of birth, gender, geographic ancestry and anthropometric measurements (height and weight) in the subject's eCRF.

6.6.4. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF.

Relevant medical history can also include any medical history that contributes to the understanding of an adverse event that occurs during study participation, if it represents an exacerbation of an underlying disease/pre-existing problem.

6.6.5. Physical examination / history directed physical examination

Perform a complete physical examination at Screening, at each vaccination visit and at the day of challenge (Day 57) and a symptom/history directed physical examination of the subject at each other visit of the vaccination and challenge phase.

The physical examination will be performed by the investigator and any outcome recorded in the subject source document. Physical examination includes assessment of oral body temperature and resting vital signs as appropriate, after at least 10 minutes of rest.

If the investigator determines that the subject's health on the days of vaccination temporarily precludes vaccination, the visit will be rescheduled within the site planning as possible. As challenge is performed per cohort, any subject with fever the day of challenge will not receive the challenge dose.

Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.6.6. Pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test at Screening visit, prior to study vaccine/placebo administration (any dose), prior to challenge and at last study visit (Visit 8). The study vaccine/placebo and challenge agent may only be administered if the pregnancy test is negative.

Note: Pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

6.6.7. Check contraindications to vaccination

Contraindications to vaccination must be checked at Screening visit and at the beginning of each vaccination visit. Refer to Section 7.5 for more details.

6.6.8. Assess pre-vaccination body temperature

The oral body temperature of each subject needs to be measured prior to any study vaccine/placebo or challenge agent administration. If the subject has fever (fever is defined as temperature ≥ 38.0°C/100.4°F) on the day of vaccination, the visit will be rescheduled within the allowed interval (see Table 5). If fever occurs at the day of challenge, the subject will not receive the challenge agent.

6.6.9. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 6.2.2. The number of each administered treatment must be recorded in the eCRF.

6.6.10. Sampling

Refer to the Module on Biospecimen Management in the Sponsor's SPM for detailed instructions for the collection, handling and processing of the samples.

6.6.10.1. Blood sampling for safety or immune response assessments

Blood samples will be taken during certain study visits as specified in Section 3 and Section 6.5. Refer to the SPM for details on samples handling, storage and shipment conditions.

- An overall volume of 400 mL will be collected per subject during the entire study period (8 months).

6.6.10.2. Urine sampling

- A urine sample for urinalysis should be collected from all subjects at each predefined timepoint, as per investigator's judgment.
- Urine samples for the pregnancy test will be collected from women of childbearing potential at each predefined timepoint (refer to Section 6.6.6).

6.6.10.3. Stool sampling

For the microbiome analysis, subjects will take a stool sample at home/at the hospital using the sampling material and instructions provided as close as possible to the study visit (preferably on the day of the visit). Immediately after stool sampling, participants will write down date and time of sampling and time since previous defecation and determine stool consistency using the Bristol Stool Score chart provided.

Aliquots of stool will be collected for fecal sIgA, inflammatory response**,** culture and qPCR, at each predefined timepoint (see Table 4). If, during the inpatient stay, no stools are passed on a given day, up to two rectal swabs can be collected for culture and qPCR (see also Section 3.2). The aliquots for fecal sIgA and inflammatory markers should be frozen at -80°C as soon as possible at the clinical site.

Refer to the SPM for more details on sample collection and storage conditions.

6.6.11. Study vaccine/placebo administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccine or placebo will be administered IM preferably in the deltoid of the non-dominant arm (refer to Section 7 for detailed description of the vaccine/placebo administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccine/placebo administration, the visit will be rescheduled within the allowed interval for this visit (refer to Table 5).
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine/placebo, with appropriate medical treatment readily available in case of anaphylaxis.

6.6.12. Challenge administration and inpatient stay

- After completing all prerequisite procedures prior to challenge, one challenge dose of S. sonnei 53G will be administered. If the investigator or delegate determines that the subject's health on the day of administration precludes challenge administration, the challenge agent will not be administrated to the subject.
- The subjects will be observed closely for the entire period of the inpatient stay (9 days, from Day 57 to Day 65) following the administration of the challenge, with appropriate medical treatment readily available in case of need.

Refer to Section 3.2 and SPM for more details related to the preparation and administration of the challenge agent and the collection of samples during the inpatient stay.

6.6.13. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 7.6.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 7.7.

6.6.14. Recording of AEs, SAEs, pregnancies and AESIs

- Refer to Section 9.3 for procedures for the investigator to record AEs, SAEs, pregnancies and AESIs. Refer to Section 9.4 for guidelines and how to report SAE, pregnancy and AESI reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

6.6.14.1. Subject Diary

A Paper Diary (pDiary), hereafter referred to as Subject Diary, will be used in this study to capture solicited adverse events. The subject should be trained on how and when to complete each field of the Subject Diary.

Subject Diary training should be directed at the individual(s) who will perform the measurements of adverse events and who will enter the information into the Subject Diary. This individual may not be the subject, but if a person other than the subject enters information into the Subject Diary, this person's identity must be documented in the Subject's source records. Any individual that makes entries into the Subject Diary must receive training on completion of the Subject Diary at the time of the visit when Subject Diary is dispensed. This training must be documented in the subject's source record.

The same individual should complete the Subject Diary throughout the course of the study.

The subject should be trained on how to self-measure local solicited adverse events and body temperature.

The measurement of solicited local adverse events is to be performed using the ruler provided by the site.

Subjects will be instructed to measure and record the oral body temperature in the evening. Should additional temperature measurements be performed at other times of day, subjects will be instructed to record the highest temperature in the Subject Diary.

6.6.14.2. Post-vaccination reminders

Reminder calls or alerts are not intended to be an interview for collection of safety data. If the subject wishes to describe safety information, this information should only be collected by a healthcare professional at the site, and the safety data described must be written down in the subject's medical chart.

6.6.14.2.1. Subject Diary reminder calls

Subject Diary reminder calls will be performed on Day 3, Day 7, Day 31 and Day 35. The purpose of this call is to remind the subject about completion of the Subject Diary. The call follows the Subject Diary Reminder Telephone Call Script provided to the site. The subject should be reminded to contact the site via the telephone number provided in the informed consent to discuss medical questions.

6.6.15. Study conclusion

The investigator will:

- Review data collected to ensure accuracy and completeness.
- Complete the Study Conclusion screen in the eCRF.

6.7. Biological sample handling and analysis

Please refer to the SPM and Investigator Manual for details on biospecimen management (handling, storage and shipment).

Samples stored at the clinical site or shipped to external laboratories will not be labelled with information that directly identifies the subject but will be coded with a unique sample identifier. Samples analyzed locally (e.g., safety lab samples) would have a label with the subject's identifiers and medical record number.

- Collected samples will be used for protocol-mandated research and purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects will be asked to give a specific consent to allow GSK or a contracted partner to use the samples for future research. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

If additional testing is performed, the marker priority ranking given in Section 6.7.4 may be changed.

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

6.7.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the per-protocol analysis (See Section 11.5.5 for the definition of analysis sets to be analyzed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

6.7.2. Biological samples

Table 6 Biological samples

Sample type (analysis) (All subjects)	Quantity	Unit	Timepoint
Blood (hematology)	~ 5	ml	scheduled
Blood (biochemistry)	~ 5	ml	scheduled
Blood (hepatitis B, hepatitis C and HIV)	~ 10	ml	scheduled
Blood (HLA-B27)	~ 10	ml	scheduled
Blood (anti *S. sonnei* LPS IgG-Screening)*	~ 5	ml	scheduled
Blood (antibody response**, SBA and Fc glycosylation)	~ 20	ml	scheduled
Blood (PBMC) for α4β7**+/-** plasmablasts response and transcriptomics) ***(Amended: 23 July 2019)***	~ 50	ml	scheduled
Urine (urinalysis)			scheduled
Stool*** (sIgA, microbiome, inflammatory markers, weight, consistency and blood assessment, culture and qPCR)			scheduled

*For local Screening-ELISA.
**Includes anti *S. sonnei* LPS IgG-ELISA
***During the challenge phase, if stool samples are unavailable, rectal swabs will be taken for culture and qPCR.
HIV: Human immunodeficiency virus
HLA: Human leukocyte antigen
ELISA: Enzyme-linked immunosorbent assay
IgG: Immunoglobulin G7
SBA: Serum bactericidal assay
Fc: Fragment crystallisable
sIgA: Secretory immunoglobulin A
qPCR: quantitative polymerase chain reaction

6.7.3. Laboratory assays

Please refer to Appendix A for a detailed description of the assays performed in the study. Please refer to Appendix B for the address of the clinical laboratories used for sample analysis.

The assays to be performed in the study are summarized in the Table 7, Table 8, Table 9 and Table 10:

Table 7 Immunity (Antibody determination)

System	Component	Method	Kit/ Manufacturer	Unit[2]	Cut-off[2]	Laboratory[3]
Serum (screening)	Anti-*S. sonnei* LPS IgG	ELISA[1]	WRAIR	Endpoint titer	plate dependent	CCHMC
Serum (except screening)	Anti-*S. sonnei* LPS IgG	ELISA	In-house	EU/mL	plate dependent	GVGH[4] or GVGH designated lab
Serum	Bactericidal antibodies	SBA	In-house	Dilution Factor (titer)	NA	GVGH[4] or GVGH designated lab
Stool	Anti-*S. sonnei* LPS **s**IgA	ELISA	WRAIR	EU/mL	10	CCHMC
Stool	Anti-total sIgA	ELISA	WRAIR	mg/mL	0.001	*CCHMC*

[1] Local Screening-ELISA will be used for this testing.
[2] Assay cut-off and unit might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization). In this case, this will be documented in the clinical report.
[3] Refer to Appendix B for the laboratory addresses.
[4] GVGH laboratory refers to the GVGH-laboratories in Siena, Italy.
ELISA: enzyme-linked immunosorbent assay, **NA**: not applicable, **EU**: ELISA unit, **LPS**: lipopolysaccharide, **IgA/G**: immunoglobulin A/G, **SBA**: serum bactericidal Assay, **WRAIR**: Walter Reed Army Institute of Research (US), **GVGH**: GSK Vaccines Institute for Global Health, **CCHMC**: Cincinnati Children's Hospital Medical Center; ***sIgA:*** secretory Immunnoglobulin A

Table 8 Cell-Mediated Immunity (CMI) (Amended: 23 July 2019)

System	Component	Method	Unit	Laboratory
PBMC	α4β7 ***positive*** plasmablast ***(Amended: 23 July 2019)***	***ELISA (Amended: 23 July 2019)***	***Anti- S. sonnei LPS specific Titer/ 5x10^6 cells (Amended: 23 July 2019)***	WRAIR or GVGH designated lab
PBMC (Amended: 23 July 2019)	***α4β7 negative plasmablast (Amended: 23 July 2019)***	***ELISA (Amended: 23 July 2019)***	***Anti- S. sonnei LPS specific Titer/ 5x10^6 cells (Amended: 23 July 2019)***	***WRAIR or GVGH designated lab (Amended: 23 July 2019)***
PBMC	Gene expression (transcriptomics) study	NextGen sequencing/ microarray	Sequencing or fluorescent intensity	GVGH* or GVGH designated lab

ELISA: Enzyme-Linked Immunosorbent Assay; **PBMC**: peripheral blood mononuclear cells, **WRAIR**: Walter Reed Army Institute of Research (US), **GVGH**: GSK Vaccines Institute for Global Health.
*GVGH laboratory refers to the GVGH-laboratories in Siena, Italy.

Table 9 Excretion of Challenge agent

System	Component Family	Method	Unit	Laboratory
Stool	*S. sonnei* 53G	culture	Not applicable	CCHMC
Stool	*S. sonnei* 53G	qPCR	RFU	WRAIR

qPCR: quantitative polymerase chain reaction, **RFU**: relative fluorescence units, **CCHMC**: Cincinnati Children's Hospital Medical Center, **WRAIR**: Walter Reed Army Institute of Research (US).

Table 10 Hematology, Serum Chemistry, Virology, HLA-B27 test, Urine and Stool tests (Amended: 23 July 2019)

System	Component	Method	Laboratory
EDTA Blood	Hematology	CBC	Study site
EDTA blood	Chemistry*	Renal and liver function test	Study site
Clotted Blood	Hep B, Hep C and HIV	TBD	Study site
EDTA Blood	HLA-B27	***Qualitative flow cytometry (Amended: 23 July 2019)***	Study site
Urine	Urinalysis	Urine dipstick (urine culture as appropriate)	Study site
Urine	Urine pregnancy test	βHCG	Study site
Stool	Microbiome	16s ribosomal DNA amplicon sequencing and/or shotgun whole genome sequencing	GVGH** or GVGH designated lab
Stool	Inflammatory markers	ELISA	WRAIR or GVGH designated lab

EDTA: Ethylene Diamine Tetra-Acetic Acid, **CBC**: Complete Blood Count, **ELISA**: Enzyme-Linked Immunosorbent Assay**, Hep**: Hepatitis, **HIV**: Human Immunodeficiency Virus, **HLA**: Human Leukocyte Antigen, **βHCG**: beta-Human Chorionic Gonadotropin, **GVGH**: GSK Vaccines Institute for Global Health, **WRAIR:** Walter Reed Army Institute of Research (US***).***
*Chemistry performed only at screening unless required for subject management
**GVGH laboratory refers to the GVGH-laboratories in Siena, Italy

Note that hematology data post vaccination/challenge will be transferred from the study laboratory performing the testing into the clinical database as soon as possible to ensure that all necessary information are available for IDMC reviews. In addition, results will be communicated as soon as possible to the investigators via lab reports, hard copies, faxes or emails.

Microbiome analysis in stool will be done using 16s ribosomal deoxyribonucleic acid (DNA) amplicon sequencing and/or shotgun whole genome sequencing. These will use DNA sequencing to identify the bacteria and their genes present in the sample to explore associations to immunogenicity and challenge outcome. Shotgun sequencing might generate subject's DNA sequence information; however, these sequences are filtered out and will not be analyzed. Depending on the results of the microbiome analysis, additional follow-up studies might include microbiome gene expression profiling (ribonucleic acid sequencing or another method), culture and functional characterization of the bacteria in the sample in animal models.

Fecal markers of intestinal inflammation will be measured as a sensitive predictor for inflammatory activities in the gastrointestinal tract. This analysis will assess if concentrations of inflammatory markers such as calprotectin and myeloperoxidase will increase after oral challenge with *S. sonnei* 53G and will additionally investigate if the *S. sonnei* 1790GAHB vaccine can reduce or abrogate the inflammatory response in the intestine after oral challenge.

Gene expression will be studied using high-throughput technologies (including but not limited to RNAseq or microarrays) measuring the relative abundance of RNA molecules in PBMCs harvested at several timepoints following vaccination and challenge. Computational analysis will subsequently identify pathways and biological themes associated with vaccination and acute disease and transcriptional patterns may be related to metadata including humoral and cellular responses to vaccination.

Additional exploratory testing on the vaccine and/or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data or should such assay(s) become available at GSK. These assays may not be represented in the objectives/endpoints of the study protocol.

Sera will be archived at GSK laboratory or its designated lab for future research on immunogenicity of the Shigella vaccine. Study-related future research may include additional evaluation of immunogenicity on *S. sonnei* (i.e., IgM, sIgA against the OAg or IgG against other antigens of *S. sonnei*, sIgA from stimulated lymphocytes supernatant, FACS/CyTOF Phenotyping (Cytokine stimulation). Non-study related future research may include serology on other Shigella serotypes and other bacteria causing infectious diseases in developing countries (e.g., *Salmonella typhi*, *Salmonella paratyphi*, non-typhoidal Salmonella, meningitis) to inform development of other vaccines relevant to the populations in developing countries.

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

6.7.4. Biological samples evaluation

6.7.4.1. Immunological read-outs

Table 11 Immunological read-outs

Sampling timepoint		Sub-cohort name	No. subjects	Component	Components priority rank
Type of contact and timepoint	Sampling timepoint				
Screening Visit	Screening	All screened subjects	NA	Anti HIV*	1
				HBsAg*	1
				Anti HCV*	1
				Serum anti-*S. sonnei* LPS IgG*	1
				HLA-B27	1
Visit 1 (Day1)	Pre-Vacc	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				Serum anti-*S. sonnei* bactericidal antibodies	2
				Serum for Fc Glycosylation	3
				α4β7 plasmablast	2
				Transcriptomics	2
		All subjects enrolled	72	sIgA in stool	NA
Visit 2 (Day 8)	Post Vacc 1	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				sIgA in stool	NA
				α4β7 plasmablast	2
				Transcriptomics	2
Visit 3 (Day 29)	Post Vacc 1	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				Serum anti-*S. sonnei* bactericidal antibodies	2
Visit 4 (36)	Post Vacc 2	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				α4β7 plasmablast	2
				Transcriptomics	2
				sIgA in stool	NA
Visit 5 (Day 57)	Pre-challenge	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				Serum anti-*S. sonnei* bactericidal antibodies	2
				Serum for Fc Glycosylation	3
Inpatient Stay (Day 64)	Inpatient Stay	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	1
				sIgA in stool	NA
				α4β7 plasmablast	2
				Transcriptomics	2
Visit 6 (Day 85)	Post-challenge	All subjects enrolled	72	Serum anti-*S. sonnei* LPS IgG	NA

Note: Anti-*S. sonnei* LPS IgG will be determined by local Screening-ELISA at screening and by ***GVGH*** ELISA for the rest of the timepoints.
*This testing is performed at screening and the results might potentially impact the subject medical care.
Vacc: Vaccination, **Fc**: Fragment crystallisable, **LPS**: Lipopolysaccharide, **IgG**: Immunoglobulin G, **α4β7**: Gut-homing integrin, **sIgA**: Secretory specific Immunoglobulin A, **IgG**: Immunoglobulin G, **HIV**: Human Immunodeficiency Virus; **HBsAg**: Hepatitis B surface Antigen; **HCV**: Hepatitis C Virus, **NA**: Not Applicable

In case of insufficient blood sample volume to perform assays, the samples will be analyzed according to priority ranking provided in Table 11.

6.7.4.2. Hematology/blood chemistry

Table 12 Hematology/Blood Chemistry read-outs

Blood sampling timepoint		No. subjects	Component
*Type of contact and timepoint	Sampling timepoint		
Screening	Pre-Vacc 1	NA	Hematology Chemistry
Visit 2 (Day 8)	Post-Vacc 1	72	Hematology
Visit 3 (Day 29)	Pre-Vacc 2	72	Hematology
Visit 4 (Day 36)	Post-Vacc 2	72	Hematology
Visit 5 (Day 57)	Pre-challenge	72	Hematology
Visit 8 (Day 237)	Post-challenge	72	Hematology

*Hematology is repeated at 7 and 28 days after each vaccination and repeated weekly until resolution if neutropenia occurs.
Vacc: Vaccination, **NA**: Not applicable

6.7.5. Immunological correlates of protection

No generally accepted immunological correlate of protection has been demonstrated so far for Shigellosis and consequently for the candidate vaccine. However, in GVGH Phase I clinical protocols a high seroresponse was defined as a post-vaccination level of ≥ 121 serum IgG EU/mL against the *S. sonnei* OAg containing LPS used as coating antigen in GVGH ELISA. This concentration corresponds to a titer of 1:800 in the ELISA method published by Cohen [Cohen, 1989] and is the median antibody concentration of a set of 87 Israeli convalescent subjects previously exposed to *S. sonnei*. The value of 121 EU/mL in the GVGH ELISA (anti-*S. sonnei* LPS IgG-ELISA) has been determined by calibration against the Cohen ELISA (i.e., the GVGH standard serum was tested in Cohen's lab using the Cohen's methodology) [Gerke, 2015].

The availability of this threshold has allowed GVGH to correlate the immunogenicity data induced by 1790GAHB vaccine with antibody levels after natural exposure in humans, which are associated with clinical protection from subsequent infections with the same *Shigella* serotype [Cohen, 1991; Ferreccio, 1991]. To confirm the proof of concept outcome of GVGH Phase I studies (post-immunization median antibody titer in vaccinees ≥ 121 EU/mL), as part of the objectives of this study, any potential correlation between antibody response (ELISA and SBA) after vaccination and likelihood of infection will be evaluated. Additionally, potential correlation between antibody response by ELISA and serum bactericidal activity will be explored. Further correlation can be established post-challenge.

7. STUDY VACCINE/PRODUCTS AND ADMINISTRATION

7.1. Description of study vaccine/placebo and challenge agent

The 1790GAHB candidate vaccine has been developed by GVGH, property of GSK Biologicals.

The *S. sonnei* 53G challenge strain was developed by Walter Reed Army Institute of Research (WRAIR), Silver Spring, Maryland, USA.

The Quality Control Standards and Requirements for the candidate vaccine/placebo and challenge agent are described in separate Quality Assurance documents (e.g., release certificate/certificate of compliance) and required approvals will be obtained before use.

The vaccine/placebo and challenge agent are labelled and packed according to applicable regulatory requirements.

Table 13 Study vaccine/placebo and challenge agent

Treatment name*	Vaccine/ placebo and challenge agent name	Formulation	Presentation	Volume to be administered	Number of doses
*S. sonnei**	Shigella sonnei 1790GAHB	200 μg 1790-GMMA protein adsorbed to 0.7 mg aluminum^{3+}/mL	White opalescent sterile suspension for injection	0.5 mL after 1:4 dilution with GVGH Placebo	2
Placebo*	GAHB-Placebo	Aluminum hydroxide (wet gel suspension *Alhydrogel* 2.0%) 0.7 mg aluminum^{3+}/mL of constituted vaccine	White opalescent sterile suspension for injection	0.5 mL	2
S. sonnei 53G challenge strain**	*S. sonnei* 53G Challenge	*S. sonnei* strain 53G	1 mL of the inoculum (CFU TBD) will be added to 30 mL of sterile normal saline 0.9% for administration	1500 CFU	1

*The supply is managed by GVGH with the support of a local Contract Manufacturing Organization.
***S. sonnei* 53G strain will be used for the challenge.

7.2. Storage and handling of study vaccine/placebo and challenge agent

The study vaccine/placebo and challenge agent must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccine/placebo and challenge agent.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of +2 to +8°C (for +2 to +8°C label storage condition) impacting 1790GAHB vaccine and placebo or -80°C ± 10°C for the challenge agent must be reported to GSK using the appropriate temperature excursion form. The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccine/placebo and challenge agent.

7.3. Dosage and administration of study vaccine/placebo and challenge agent

7.3.1. Vaccine/placebo preparation

Bedside mixing and vaccine/placebo administration at the study site will be performed according to the Sponsor instructions by trained site staff. The site staff responsible for these activities will be personnel who are qualified according to applicable laws and regulations and will not be involved in the clinical assessment of the subjects. The Sponsor, or its authorized delegate, will provide specific procedures and training for these activities.

7.3.2. Preparation of challenge agent

Preparation and administration of the challenge agent at the study site will be performed according to study site instructions by trained site staff. The site staff responsible for these activities will be personnel who are qualified according to applicable laws and regulations. *S. sonnei* 53G is classified as a BSL-2 category organism. Personnel will take appropriate precautions for working with BSL-2 organisms and wear proper protective equipment such as lab coats, gloves and, if necessary, masks to shield themselves and others from spills and inadvertent mishaps. Spills will be treated according to Standard Operating Procedure (SOP). All unused reconstituted inoculum and materials containing the bacteria will be disposed of properly in biohazard bags. All exposed materials and all disposable materials will be discarded in the biohazard waste for pick up and decontamination as per CCHMC policy. At the study site specific procedures are available and site staff will be appropriately trained.

7.3.3. Vaccine/placebo dosage and administration

The investigator or designee will be responsible for the oversight of the administration of vaccine/placebo to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines/placebos will be administered only by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

7.3.4. Challenge agent dosage and administration

The investigator or designee will be responsible for the oversight of the administration of the challenge agent to subjects enrolled in the study according to the procedures stipulated at the study site. The challenge agent will be administered only by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

Table 14 Dosage and administration

Type of contact and timepoint	Study group	Treatment name	Volume to be administered	Route[1]	Site		
					Location	Directionality[2]	Laterality[3]
Visit 1 (Day 1)	*S. sonnei*	GVGH 1790GAHB *S. sonnei* vaccine (1:4 diluted)	0.5 mL	IM	Deltoid	Upper	Non-dominant
	Placebo	GAHB-Placebo	0.5 mL	IM	Deltoid	Upper	Non-dominant
Visit 3 (Day 29)	*S. sonnei*	GVGH 1790GAHB *S. sonnei* vaccine (1:4 diluted)	0.5 mL	IM	Deltoid	Upper	Non-dominant
	Placebo	GAHB-Placebo	0.5 mL	IM	Deltoid	Upper	Non-dominant
Visit 5 (Day 57)	*S. sonnei*	*S. sonnei* 53G	1500 CFU	O	NA	NA	NA
	Placebo	*S. sonnei* 53G	1500 CFU	O	NA	NA	NA

[1] **IM**: intramuscular / **O**: oral
[2] Directionality is a qualifier for further detailing the location of the vaccine/placebo administration
[3] The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine/placebo in the non-dominant arm, an injection in the dominant arm may be performed.

Precautions to be observed in administering study vaccine/placebo or challenge agent:

Prior to administration of the vaccine/placebo or challenge agent, subjects must be determined to be eligible and it must be clinically appropriate in the judgment of the investigator to vaccinate/challenge. Eligibility for vaccination/challenge is determined by evaluating the entry criteria outlined in protocol Sections 5.2, 5.3, 6.6.11 and 6.6.12.

Study vaccine/placebo or challenge agent should not be administered to individuals with known hypersensitivity to any component of the vaccine/placebo or challenge agent. Standard immunization practices are to be observed and care should be taken to administer the vaccine/placebo intramuscularly. Before administering vaccine/placebo, the vaccination site is to be disinfected with a skin disinfectant (e.g., 70% alcohol). Allow the skin to dry. **DO NOT inject intravascularly**.

As with all injectable vaccines/products, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccine/placebo administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

7.4. Replacement of unusable vaccine/placebo doses and challenge agent

In addition to the vaccine/placebo doses provided for the planned number of subjects (including over-randomization when applicable), at least 5% additional vaccine/placebo doses will be supplied to replace those that are unusable. For the challenge agent, at least one back-up vial will be required.

The investigator will use SBIR to obtain the replacement vial number. The replacement numbers will be allocated by dose. The system will ensure, in a blinded manner that the replacement vial matches the formulation the subject was assigned to by randomization.

7.5. Contraindications to subsequent vaccination or challenge

Eligibility to administration of a second dose of the vaccine and challenge agent should be reconfirmed. The following events constitute absolute contraindications to further administration of 1790GAHB vaccine or the placebo and the challenge agent. If any of these events occur during the study, the subject must not receive additional doses of vaccine/placebo and challenge agent but may continue other study procedures at the discretion of the investigator (see Section 9.5).

- Anaphylaxis following the administration of vaccine/placebo.
- Pregnancy (see Section 9.2.1).
- Hepatomegaly, right upper quadrant abdominal pain or tenderness.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including, HIV infection.
- Any condition that in the judgment of the investigator would make intramuscular injection unsafe.

The following event*s* constitute contraindications to administration of 1790GAHB vaccine or the challenge agent at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 6.5), or the subject may be withdrawn at the discretion of the investigator (see Section 9.5):

- Neutropenia below 1800 cell/µL (LLN) based on most recent available laboratory results. Subjects with previous neutropenia or unresolved neutropenia may be re-evaluated prior to vaccination or prior to challenge at the discretion of the investigator.
- Acute disease and/or fever at the time of vaccination.
  - Fever is defined as temperature ≥ 38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
  - Subjects with a minor illness (such as mild diarrhea, mild upper respiratory infection) without fever can be administered vaccine/placebo and challenge agent.

7.6. Concomitant medications/products and concomitant vaccinations

At each study visit/contact, the investigator or delegate should question the subject about any medications/products taken and vaccinations received by the subject.

7.6.1. Recording of concomitant medications/products and concomitant vaccinations

The following concomitant medication(s)/product(s)/vaccine(s) must be recorded in the eCRF:

- All concomitant medications/products, except vitamins and dietary supplements, administered during the period starting at vaccination day to 28 days following each dose of study vaccine/placebo (1 day to 28 days after each vaccination) and administration of challenge agent.
- Relevant medications/products like vaccines, immunoglobulin, blood products, immunosuppressors, immunomodulators administered during the period starting from the administration of the first dose of the study vaccine/placebo and ending 28 days after challenge.
- Any concomitant vaccination administered in the period starting 28 days before the first dose of study vaccine/placebo and ending at 28 days after challenge with the exception of seasonal Flu vaccination.
- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g., an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring (fever is defined as temperature ≥ 38.0°C/100.4°F*)*. The preferred location for measuring temperature in this study will be the oral cavity.
- Any concomitant medications/products/vaccines relevant to a SAE to be reported as per-protocol or administered during the study period for the treatment of a SAE. In addition, concomitant medications relevant to SAEs need to be recorded on the expedited Adverse Event report.

7.6.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from per-protocol analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See Section 11.5 for analyses sets to be analyzed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccine/products used during the study period.

- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days in total) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day or equivalent. Inhaled, except for doses >800 µg/day, and topical steroids are allowed.
- Long-acting immune-modifying drugs administered at any time during the study period (e.g. infliximab).
- A vaccine not foreseen by the study protocol administered during the period starting from the first dose and ending at the blood sample at Visit 8 or 28 days after the last dose of vaccine/placebo administration, with the exception of emergency mass vaccination and influenza vaccination.

  In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organized by the public health authorities, outside the routine immunization program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its Summary of Product Characteristics or Prescribing Information and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.
- Immunoglobulins and/or any blood products administered during the study period.
- Drug and/or alcohol abuse during the study period.

7.7. Intercurrent medical conditions that may lead to elimination of a subject from per-protocol analyses

At each study visit subsequent to the first vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the per-protocol cohort for immunogenicity if, during the study:

- They incur a condition that has the capability of altering their immune response (i.e., varicella) or are confirmed to have an alteration of their initial immune status.
- They have serious chronic or progressive disease according to judgment of the investigator (e.g., neoplasm, insulin dependent diabetes, cardiac, renal or hepatic disease).
- They have any malignancy or lymphoproliferative disorder.
- They have any confirmed or suspected immunosuppressive or immunodeficient condition, based on physical examination.

8. HEALTH ECONOMICS

Not applicable.

9. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they/the subject manifest any signs or symptoms they perceive as serious.

9.1. Safety definitions

9.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study vaccine/placebo administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study vaccine/placebo or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with study vaccine/placebo.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

AEs to be recorded as endpoints (solicited AEs) are described in Section 9.1.3. All other AEs will be recorded as UNSOLICITED AEs.

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital, admission for routine examination).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination. These events will be recorded in the medical history section of the eCRF.

9.1.2. Definition of a serious adverse event

A SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalization or prolongation of existing hospitalization,

   Note: In general, hospitalization signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an outpatient setting. Complications that occur during hospitalization are also considered AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

   Hospitalization for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, OR

   Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza like illness, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect in the offspring of a study subject.

Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization.

9.1.3. Solicited adverse events

The term "reactogenicity" refers to solicited signs and symptoms ("solicited AEs") occurring in the hours and days following a vaccination, to be collected by the subjects for 7 consecutive days, using a predefined Subject Diary.

The study staff must review the data entered into the Subject Diary as described in Section 12.2.

Note: Any solicited AE that meets any of the following criteria must be entered into subjects' source document (see Section 12.2) and also as an AE on the Adverse Event CRF:

- Solicited local or systemic AE that continues beyond day 7 after vaccination.
- Solicited local or systemic AE that leads to a visit to a healthcare provider (medically attended AE, see Section 9.3.3.4).
- Solicited local or systemic AE leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (AE leading to withdrawal).
- Solicited local or systemic AE that otherwise meets the definition of a SAE (see Section 9.1.2).

Solicited AEs after vaccination are included in the Subject Diary. Each AE is to be assessed using the scoring system reported in Section 9.3.3.2.1.

9.1.3.1. Solicited local Adverse events

The following local (injection site) AEs will be solicited:

Table 15 Solicited local adverse events

Both study arms
Pain at injection site
Erythema at injection site
Induration at injection site

9.1.3.2. Solicited systemic Adverse events

The following systemic AEs will be solicited:

Table 16 Solicited systemic adverse events

Both study arms
Arthralgia
Chills
Fatigue
Malaise
Myalgia
Fever
Headache

Note: Subjects will be instructed to measure and record oral body temperature in the evening 6 hours after vaccination and daily during the following 6 days. Subjects will be instructed to record the highest temperature in the diary card.

9.1.3.3. Solicited events after challenge phase

During the challenge phase after the subjects have received the challenge agent, events associated with the administration of the challenge agent will be collected and recorded by the investigator in the CRF. These events include diarrhea, abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, and vomiting beginning the day of administration of study challenge agent until 7 days after challenge (i.e., Day 64). Any unresolved events will be followed to resolution or stabilization. Oral rehydration will be initiated and recorded for subjects with a Grade 2 or higher AE of diarrhea, vomiting, dysentery or dehydration. The hydration of subjects developing diarrhea will be maintained with oral electrolyte solutions by offering at least 1.5 mL for each gram of diarrheal stool lost, as tolerated. Subjects who develop vomiting will be offered at least 1.0 mL for each gram of emesis lost, as tolerated. Subjects will be evaluated by a physician who will consider intravenous hydration for subjects who:

- Cannot tolerate oral fluids,
- Experience fluids loss that exceeds their ability to drink replacement fluids,
- Have > 1000 mL deficit in intake in a 24 hour period,
- Experience weight loss of more than 5%,
- Have urine specific gravity > 1.030 for 12 hours,
- Have syncope or near-syncope,
- Grade 3 tachycardia, or
- Grade 3 hypotension.

If the subject is unable to tolerate fluids by mouth and intravenous fluids are deemed necessary by the assessing physician, a 0.9% sterile saline solution 1-liter bolus will be administered intravenously. This bolus will be repeated as needed to resolve Grade 2 and above symptoms at the discretion of assessing physician. Fluid maintenance will be continued using 0.45% NaCl, 5% dextrose, and 20 mEq KCL per liter at a rate of 100 mL/hour until subject is able to resume oral rehydration. If the subject develops grade 4 hypotension despite the aggressive fluid management, the subject will be transferred from the inpatient unit to a hospital setting.

9.1.4. Unsolicited Adverse Events

An unsolicited AE is an AE that was not solicited using a Subject Diary and that was spontaneously communicated by a subject who has signed the informed consent or a solicited local or systemic AE that continues beyond the solicited period at day 7 after vaccination.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a health care provider), or were of concern to the subjects. In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified site personnel during the interview and will be documented in the subject's records.

Unsolicited AEs that are not medically attended nor perceived as a concern by subjects will be collected during interview with the subjects and by review of available medical records at the next visit.

Unsolicited AEs will be collected until 28 days after each vaccination and until 28 days after challenge.

9.1.5. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections 9.1.1 and 9.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant modifications in hematology will be assessed by medical judgment based on interpretation of deviations from institution's normal values and recommendations from CBER FDA GUIDANCE FOR INDUSTRY: Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials and predefined list of sign and symptoms related to neutropenia.

9.1.6. Adverse events of special interest

Adverse events of special interest (AESIs) are predefined (serious or non-serious) AEs of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate, because such an event might warrant further investigation in order to characterize and understand it.

During the two Phase I studies with the study vaccine, no SAE were reported. Two subjects of African descent experienced a transient and clinically asymptomatic Grade 3 neutropenia (the two episodes were graded as SAEs) that was finally classified as "benign ethnic neutropenia". In addition to these two Grade 3 cases (one in each study), a total of 6 cases of transient and clinically asymptomatic Grade 2 neutropenia (one case in H03_01TP and 5 cases in H03_02TP) has been reported. GVGH has defined neutropenia as AESI. In the phase 2 study in endemic population, there have been 10 cases of neutropenia from 6 subjects of which one was severe, three moderate and 6 mild. All of these events were clinically asymptomatic.

During the course of this study, symptomatic neutropenia will be considered as AESIs.

Table 17 List of potential AESI to be followed during the study

Blood disorders
Symptomatic neutropenia (all Grades)

Neutropenia is defined as a decrease of neutrophil count asymptomatically or symptomatically. This is completely diagnosed by laboratory testing for complete blood count. However, only symptomatic neutropenia cases will be considered as AESI and reported as such.

The following grading will be used to classify neutropenia:

- Grade 1: 1800 -1500 cells/µL.
- Grade 2: 1499-1000 cells/µL.
- Grade 3: 999-500 cells/µL.
- Grade 4: < 500 cells/µL.

In performing their assessment of symptomatic neutropenia cases, investigators are strongly advised to use the table below.

Table 18 Evaluation of symptomatic neutropenia

Common presenting symptoms of neutropenia	Physical findings on examination of a patient with neutropenia
Low-grade fever	Fever
Sore mouth	Stomatitis
Odynophagia	Periodontal infection
Gingival pain and swelling	Cervical lymphadenopathy
Skin abscesses	Skin infection: The skin examination focuses on rashes, ulcers, or abscesses
Recurrent sinusitis and otitis	Splenomegaly
Symptoms of pneumonia (e.g., cough, dyspnea)	Associated petechial bleeding
Perirectal pain and irritation	Perirectal infection
Neutropenic sepsis	Other according to Investigator's opinion
Neutropenic infection	
Neutropenic colitis	
Other according to Investigator's opinion	

In order to facilitate the documentation of AESI in the eCRF, a list of MedDRA preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as AESI. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as AESI until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

9.2. Events or outcomes not qualifying as adverse events or serious adverse events

9.2.1. Pregnancy

Female subjects who become pregnant before the 2nd vaccination and before the challenge must not receive additional doses of study vaccine/placebo, nor the challenge agent, but may continue other study procedures at the discretion of the investigator.

While pregnancy itself is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

Note: The pregnancy itself should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 9.4.1 and 9.4.3:

- Spontaneous pregnancy loss, including:
  - Spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - Ectopic and molar pregnancy
  - Stillbirth (intrauterine death of fetus after 22 weeks of gestation).

  Note: the 22 weeks cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the study vaccine/placebo or challenge agent will be reported to GSK Biologicals as described in Section 9.4.3. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

9.3. Detecting and recording adverse events, serious adverse events and pregnancies

9.3.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

All AEs starting within 28 days following administration of each dose of study vaccine/placebo (day of vaccine/placebo administration to 28 days after administration) and challenge agent administration must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The time period for collecting and recording SAEs will begin at the first receipt of study vaccine/placebo and will end at the conclusion of the study for each subject. See Section 9.4 for instructions on reporting of SAEs.

In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study.

The time period for collecting and recording pregnancies will begin at the first receipt of study vaccine/placebo and will end at the conclusion of the study. See Section 9.4 for instructions on reporting of pregnancies.

The time period for collecting and recording of AESIs will begin at the first receipt of study vaccine/placebo and will end at the conclusion of the study. See Section 9.4 for instructions on reporting of AESIs.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given in Table 19.

Table 19 Reporting periods for collecting safety information

Timepoints	Scr	D1	D8	D29	D36	D57	D57-D64	D85	D237 (M8)
Solicited local and systemic AEs									
Unsolicited AEs									
AEs/SAEs leading to withdrawal from the study									
AESI SAEs									
Pregnancies									
SAEs related to study participation or concurrent GSK medication/vaccine									

Scr: Screening
D: Day
M: Month

9.3.2. Post-study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 19. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the study vaccine/placebo or challenge agent, the investigator will promptly notify the Study Contact for Reporting SAEs.

9.3.3. Evaluation of adverse events and serious adverse events

9.3.3.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as:

'*Have you felt different in any way since receiving the vaccine/placebo or since the previous visit?*'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

9.3.3.2. Assessment of adverse events

9.3.3.2.1. Assessment of intensity

The intensity of the following solicited AEs will be assessed as described:

Table 20 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
After vaccine/placebo administration		
Pain at injection site	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Erythema at injection site		Record greatest surface diameter in mm
Induration at injection site		Record greatest surface diameter in mm
Fever#*		Record temperature in °C/°F
Headache#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Fatigue#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Arthralgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Malaise#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Myalgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Chills	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
After challenge agent administration		
Nausea	0	Absent
	1	Mild or transient; maintains reasonable intake
	2	Moderate discomfort; intake decreased significantly; some activity limited
	3	No significant intake and requires medical intervention
	4	Hospitalization required
Abdominal cramping	0	Absent
	1	No interference with daily activities
	2	Some interference with daily activities not requiring medical intervention
	3	Prevents daily activities and requires medical intervention
	4	ER visit or hospitalization
Abdominal pain	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Gas	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity

Adverse Event	Intensity grade	Parameter
Anorexia	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Vomiting	0	Absent
	1	One episode within a 24-hour period Vomiting
	2	2 episodes within a 24-hour period
	3	>2 episodes within a 24-hour period and requires medical intervention
	4	ER visit or hospitalization for hypotensive shock
Diarrhea**	0	Absent
	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools (loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours or requires medical intervention
	4	≥ 10 loose stools (Grade 3 to 5) or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours

#Same grading is used for events present both post-vaccination and post-challenge.
*Fever is defined as temperature ≥ 38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
**The end of a diarrheal episode occurs when a volunteer does not pass any Grade 3-5 stool within 24 hours.
ER: emergency room.

The maximum intensity of local injection-site erythema/induration/fever will be scored at GSK Biologicals as follows:

	Erythema/Induration
0:	< 25 mm
1:	≥ 25 - ≤ 50 mm
2:	> 50 - ≤ 100 mm
3:	> 100 mm

Fever			
Grade 0 Absent	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**
≤ 37.9°C	≥ 38.0°C – 38.9°C	≥ 39.0°C – 39.9°C	≥ 40.0°C

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgment.

Every effort should be made by the investigator to evaluate safety information reported by a subject for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., "cough" or "ear pain") are better reported according to the underlying cause (e.g., "asthma exacerbation" or "otitis media").

The severity of events reported on the Adverse Events eCRF will be determined by the investigator as:

Mild:	Transient with no limitation in normal daily activity
Moderate:	Some limitation in normal daily activity
Severe:	Unable to perform normal daily activity

9.3.3.2.2. Assessment of causality

Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the study vaccine/placebo or challenge agent will be considered and investigated. The investigator will also consult the IB to determine his/her assessment.

There may be situations when a SAE has occurred, and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the Expedited Adverse Events Report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

All solicited reactions will be considered causally related to vaccination or challenge. Causality of all other AEs should be assessed by the investigator using the following question:

Is there a reasonable possibility that the AE may have been caused by the study vaccine/placebo or challenge agent?

YES: There is a reasonable possibility that the study vaccine/placebo or challenge agent contributed to the AE.

NO: There is no reasonable possibility that the AE is causally related to the administration of the study vaccine/placebo or challenge agent. There are other, more likely causes and administration of the study vaccine/placebo or challenge agent is not suspected to have contributed to the AE.

If an event meets the criteria to be determined as ‘serious’ (see Section 9.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol-required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccine, if applicable.
- Erroneous administration.
- Other cause (specify).

9.3.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

9.3.3.4. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he/she received medical attention defined as hospitalization, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF.

9.4. Reporting of serious adverse events, pregnancies, and other events

9.4.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 21, once the investigator determines that the event meets the protocol definition of a SAE. Time of onset of SAE will allow distinguishing whether the SAE happened before or after challenge including any possible exacerbation of events following challenge.

Pregnancies that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 21, once the investigator becomes aware of the pregnancy.

AESIs that occur in the time period defined in Section 9.3 will be reported promptly to GSK within the timeframes described in Table 21, once the investigator determines that the event meets the protocol definition of an AESI.

Table 21 Timeframes for submitting serious adverse event, pregnancy and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report
Pregnancies	2 weeks*	electronic pregnancy report	2 weeks*	electronic pregnancy report
AESIs	24 hours**‡	electronic Expedited Adverse Events Report	24 hours*	electronic Expedited Adverse Events Report

*Timeframe allowed after receipt or awareness of the information.
**Timeframe allowed once the investigator determines that the event meets the protocol definition of a AESI.
‡The investigator will be required to confirm review of the SAE/AESI causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE/AESI.

9.4.2. Contact information for reporting serious adverse events, pregnancies and AESIs

Study Contact for Reporting SAEs, AESIs and pregnancies
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs, AESIs and pregnancies
24/24 hour and 7/7 day availability: **GSK Biologicals Clinical Safety & Pharmacovigilance** US sites only: Email address: PPD Fax: PPD

9.4.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

9.4.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designate) must complete, then date and sign a paper Expedited Adverse Events Report and fax it to the

Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designate) must complete the electronic Expedited Adverse Events Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

9.4.4. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a subject is pregnant, the investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 2 WEEKS.

Note: Conventionally, the estimated gestational age (EGA) of a pregnancy is dated from the first day of the last menstrual period (LMP) of the cycle in which a woman conceives. If the LMP is uncertain or unknown, dating of EGA and the estimated date of delivery (EDD) should be estimated by ultrasound examination and recorded in the pregnancy report.

9.4.5. Reporting of AESIs to GSK Biologicals

Once an AESI is diagnosed (serious or non-serious) in a study subject, the investigator (or designate) must complete the information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS after he/she becomes aware of the diagnosis. The report allows to specify that the event is an AESI and whether it is serious or non-serious. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding an AESI, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report. The investigator will be required to confirm the review of the AESI causality by ticking the ‘reviewed’ box in the electronic Expedited Adverse Events Report within 72 hours of submission of the AESI.

Refer to Section 9.4.3.1 for back-up system in case the electronic reporting system does not work.

9.4.6. Updating of SAE, pregnancy, and AESI information after removal of write access to the subject's eCRF

When additional SAE, pregnancy, or AESI information is received after removal of the write access to the subject's eCRF, new or updated information should be recorded on the appropriate paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the Study Contact for Reporting SAEs (refer to the Sponsor Information) or to GSK Biologicals Clinical Safety and Pharmacovigilance department within the designated reporting time frames specified in Table 21.

9.4.7. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 9.4.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the study vaccine/placebo or challenge agent and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

9.5. Follow-up of adverse events, serious adverse events, and pregnancies

9.5.1. Follow-up of adverse events and serious adverse events

9.5.1.1. Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 21).

All SAEs and AESIs (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the last visit of the subject.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

9.5.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- With SAEs, AESIs (serious or non-serious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.
- With other non-serious neutropenia cases, until the event is otherwise explained or they are lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper/electronic Expedited Adverse Events Report and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

9.5.2. Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK Biologicals using the electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period doesn't need to be longer than six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

9.6. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of a SAE/AESIs should be recorded in Expedited Adverse Event Report of the subject's eCRF (refer to Section 7.6).

9.7. Unblinding

GSK Biologicals' policy (which incorporates ICH E2A guidance, EU Clinical Trial Directive and US Federal Regulations) is to unblind the report of any SAE which is unexpected and attributable/suspected to be attributable to the study vaccine/placebo or challenge agent, prior to regulatory reporting. The GSK Biologicals' Central Safety Physician is responsible for unblinding the treatment assignment in accordance with the specified timeframes for expedited reporting of SAEs (refer to Section 9.4.1).

9.8. Emergency unblinding

Unblinding of a subject's individual treatment code should occur only in the case of a medical emergency, or in the event of a serious medical condition, when knowledge of the treatment is essential for the clinical management or welfare of the subject, as judged by the investigator.

The emergency unblinding process consists of an automated system SBIR that allows the investigator to have unrestricted, immediate and direct access to the subject's individual study treatment.

The investigator has the option of contacting a GSK Biologicals' On-call Central Safety Physician (or Backup) if he/she needs medical advice or needs the support of GSK to perform the unblinding (i.e. he/she cannot access the automated Internet-based system).

Any emergency unblinding must be fully documented by using the Emergency Unblinding Documentation Form, which must be appropriately completed by the investigator and sent within 24 hours to GSK Biologicals.

GSK Biologicals' Contact information for Emergency Unblinding **24/24 hour and 7/7 day availability**
GSK Biologicals' Central Safety Physician: For US/Canada only: PPD (GSK Biologicals Central Safety Physician on-call) **GSK Biologicals' Central Safety Physician Back-up:** US/Canada only: PPD **Emergency Unblinding Documentation Form transmission:** US/Canada only: Fax: PPD

9.9. Subject card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects must be instructed to keep subject cards in their possession at all times during the study duration.

9.10. Holding rule and safety monitoring

9.10.1. Holding rule

The following holding rule has been defined for the study:

- At least one subject developing a ≥ Grade 3 symptomatic neutropenia considered possibly, probably or definitely related to vaccination.

The Investigator must notify GVGH/GSK immediately if the holding rule is met and should halt any further vaccination until further notice. Vaccination can only be resumed after formal review of unblinded data by the IDMC and formal greenlight to proceed with further vaccination (please refer to the IDMC charter).

9.10.2. Safety monitoring

The SRT will monitor the safety of subjects throughout the study. The SRT will review blinded data, assess safety signals and make recommendations to the IDMC and to the Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects.

An IDMC will be established by GSK Biologicals for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The IDMC will provide recommendations about stopping, holding, continuing or modifying the trial.

The frequency of IDMC sessions and other operational details are described in the IDMC charter. In case of a serious safety issue during the study, GSK Biologicals will inform the IDMC as well as fulfil its regulatory obligation expeditiously.

10. SUBJECT COMPLETION AND WITHDRAWAL

10.1. Subject completion

A subject who returns for the concluding visit foreseen in the protocol is considered to have completed the study.

10.2. Subject withdrawal

Withdrawals will not be replaced.

10.2.1. Subject withdrawal from the study

From an analysis perspective, a ‘withdrawal’ from the study refers to any subject who did not come back for the concluding visit foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up.

Information relative to the withdrawal will be documented in the eCRF. The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event.
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject, in the eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 9.5.1.2).

10.2.2. Subject withdrawal from study vaccine/placebo or challenge agent

A 'withdrawal' from the study vaccine/placebo or challenge agent refers to any subject who does not receive the complete treatment, i.e. when no further planned dose or challenge is administered from the date of withdrawal. A subject withdrawn from the study vaccine/placebo or challenge agent may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the study vaccine/placebo or challenge agent will be documented on the Vaccine Administration page/screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/challenge administration was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Unsolicited non-serious adverse event.
- Solicited adverse event.
- Not willing to be vaccinated or to receive the challenge agent.
- Other (specify).

11. STATISTICAL METHODS

11.1. Primary endpoint

Efficacy

VE will be evaluated with:

- Rate of shigellosis (fulfilling the protocol primary case definition) occurring within a period starting with the challenge visit and lasting up to the end of the inpatient stay, in all subjects.

11.2. Secondary endpoints

Efficacy

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured during inpatient stay (Day 57 to Day 64) against:

- Rate of shigellosis ***as defined by*** the CHIM working group case definition for shigellosis occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects. ***(Amended: 23 July 2019)***
- Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].
- Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom]]. ***(Amended: 23 July 2019)***
- Shedding of *S. sonnei* strain 53G.

  Shedding of *S. sonnei* strain 53G is defined as positivity of at least one stool sample either by culture or qPCR or both.
- Severe diarrhea.
- More severe diarrhea.
- Dysentery.

- Weight of all grade 3-5 stools.
- Total number of all grade 3-5 stools.
- Confirmed S. sonnei 53G shedding AND moderate or severe diarrhea OR dysentery *OR* presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, anorexia).
- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- Time to onset of shigellosis after challenge, according to the protocol primary case definition.

Safety

Solicited adverse events

- Occurrence of each solicited local and systemic adverse event within 7 days after each vaccination.

Unsolicited adverse events

- Occurrence of unsolicited AEs within 28 days after vaccination and after challenge, according to Medical Dictionary for Regulatory Activities (MedDRA) classification.

Serious adverse events

- Occurrence of all SAEs and related SAEs from Day 1 to study end.

Adverse events of special interest

- Occurrence of AESI (i.e., symptomatic neutropenia) from Day 1 to study end.

Laboratory parameters

- Out of laboratory reference range and/or clinically significant value (according to local ranges) for hematology at 7 days after first and second vaccination and at last study visit (Visit 8).

Immunogenicity

The measures of immunogenicity, against the LPS of *S. sonnei*, will include:

- IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 7 and 28 days after first and second vaccination by antibody concentration at baseline (i.e., above vs. below the assay detection limit), as determined by anti-*S. sonnei* LPS IgG ELISA.
- IgG GMC pre-challenge (Day 57), 7 and 28 days after challenge (Day 64 and Day 85) as determined by anti-*S. sonnei* LPS IgG ELISA.
- Number and percentage of subjects achieving a post vaccination anti- S. Sonnei LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.

- Number and percentage of seroresponders***** for anti-*S. sonnei* LPS at 28 days after first and second vaccination.

  *****Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. For the purpose of this study seroresponse is defined as:

  – If the baseline value is greater than 50 EU/mL then an increase of at least 50% in the post-vaccination sample as compared to baseline.

  – If the baseline value is less or equal to 50 EU/mL then an increase of at least 25 EU/mL in the post-vaccination sample as compared to baseline.

  Note: Threshold values/increases given in the definition of seroresponse might be subject to change during the course of the study (e.g., in case of optimization/qualification/validation of the anti S. sonnei LPS IgG ELISA).

11.3. Tertiary endpoints

- Specific anti-*S. sonnei* LPS sIgA antibody concentration in stool samples at Day 1, 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Frequency of *S. sonnei* LPS specific IgG α4β7+**/-** antibody secreting cells per 10^6 PBMC plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 8, Day 36 and Day 64). ***(Amended: 23 July 2019)***
- Glycosylation profiles of IgG antibodies at Day 1 and 28 days after second vaccination (Visit 5; *Day 57).*
- Number and percentage of subjects that show correlation between serum anti-*S. sonnei* LPS IgG concentration, SBA titer, shigellosis, shedding, MSD, dysentery and mild diarrhea after challenge.
- Concentration of stool markers of intestinal inflammation (e.g. calprotectin and myeloperoxidase) before oral challenge with *S. sonnei*, 53G at Visit 5 (Day 57) and daily during the post-challenge inpatient stay (Day 57 to Day 64).
- The number of differential expressed genes 7 days after first and second vaccination and 7 days after challenge by comparing the relative abundance of mRNA sequences compared to Day 1 (pre-vaccination baseline).
- Diversity and frequency of microbiome components (taxa and/or genes according to technology) at Day 1 prior to vaccination and 28 days after second vaccination (Visit 5; Day 57)**.**

  Exploratory analysis for hypotheses generation will include the evaluation of the association of the microbiome components with efficacy and immunogenicity described above.

11.4. Determination of sample size

Primary objective - Efficacy

In comparing the protective efficacy of 1790GAHB vaccine against the placebo group the following assumptions will be made:

Assuming a true VE of 70%, a total number of 21 confirmed cases will be needed to demonstrate that the LL of the two-sided 90% CI for the VE is above 0% with 80% power (by Pass 2013, One Proportion Power Analysis, one-sided test, one-sided alpha = 5%).

Based on the results obtained by the Cincinnati Children's Hospital Medical Center, where the challenge model was developed, in subjects receiving a challenge with 1500 CFU of *S. sonnei* 53G, the attack rate for the primary case definition of this study is 58%. Thus considering an AR of 58% in the placebo group and a percentage of non-evaluable subjects of 22%, approximately 72 subjects (36 per group) will be recruited to reach these 21 cases.

The assumption on the value of true VE is based on the percentage of subjects with seroresponse observed in previous studies where the 1.5/25 µg OAg/protein dose of 1790GAHB vaccine was evaluated. In the phase I study in Europe, the observed percentage of subjects with seroresponse was 63% and 88% after 1st and 2nd vaccination respectively. In the Phase IIa study, conducted in endemic region, the observed percentage of subjects with seroresponse was 68% and 90% after 1st and 2nd vaccination, respectively.

11.5. Analysis sets

11.5.1. All Enrolled Set

All screened subjects who provide informed consent, and provide demographic and/or baseline screening assessments, regardless of the subject's randomization and treatment status in the study and received a Subject ID.

11.5.2. All Exposed Set

All subjects in the enrolled set who receive a study vaccination.

11.5.3. Safety set

11.5.3.1. Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set with any solicited AE data and/or indicators of solicited AEs.

11.5.3.2. Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set with unsolicited AE data.

11.5.3.3. Overall safety set

All subjects who are in the Solicited Safety Set and/or Unsolicited Safety Set.

Subjects will be analyzed as "treated" (i.e., according to the vaccine* a subject received, rather than the vaccine* to which the subject may have been randomized).

*Vaccine refers to study vaccine or placebo.

11.5.4. Full analysis set (FAS) efficacy/Immunogenicity set

11.5.4.1. Full analysis set efficacy

All subjects in the All Enrolled Set who are randomized, receive a study vaccination and provide efficacy data.

11.5.4.2. Full analysis set Immunogenicity

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and provide immunogenicity data at the relevant timepoints.

In case of vaccination error, subjects in the FAS sets will be analyzed "as randomized" (i.e., according to the vaccine* a subject was designated to receive, which may be different from the vaccine* the subject actually received).

*Vaccine refers to study vaccine or placebo.

11.5.5. Per-protocol (PP) set for efficacy/Immunogenicity set

All subjects in the FAS Efficacy / Immunogenicity who:

- Correctly receive the vaccine* (i.e., receive the vaccine* to which the subjects is randomized and at the scheduled timepoints).

  *Vaccine refers to study vaccine or placebo.
- Have no protocol deviations leading to exclusion as defined prior to unblinding / analysis.
- Are not excluded due to other reasons defined prior to unblinding or analysis.

PPS are subsets of FAS and should be always defined even if the objectives do not require it.

Examples for subjects excluded due to other reasons than protocol deviations are:

- Subjects who withdrew informed consent.

The primary analysis will be based on the PP cohort for efficacy and immunogenicity. However efficacy and immunogenicity analyses will be performed on both FAS and PP sets.

11.5.6. Other analysis sets

There are no additional analysis sets for analysis in this study.

All subjects who are screened, i.e., consented but not yet randomized/enrolled.

11.5.7. Sub-groups

The analysis for the primary efficacy endpoint will be repeated for the two subgroups of subjects who were responders or non-responders at the immunogenicity assessment of the pre-challenge visit.

11.6. Derived and transformed data

- Screening (anti *S. sonnei* LPS IgG Screening-ELISA)
  - The cut-off value for the anti *S. sonnei* LPS IgG Screening-ELISA is defined by the laboratory.
  - A seronegative subject is a subject whose titer is below the cut-off value.
  - A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.
- Immunogenicity (anti *S. sonnei* LPS IgG-ELISA):
  - The cut-off value for the anti *S. sonnei LPS* IgG-ELISA is defined by the laboratory.

  Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. For the purpose of this study seroresponse is defined as:
  - If the baseline value is greater than 50 EU/mL then an increase of at least 50% in the post-vaccination sample as compared to baseline.
  - If the baseline value is less or equal to 50 EU/mL then an increase of at least 25 EU/mL in the post-vaccination sample as compared to baseline.

  Note: Threshold values/increases given in the definition of seroresponse might be subject to change during the course of the study (e.g., in case of optimization/qualification/validation of the anti *S. sonnei* LPS IgG ELISA).
  - The GMC calculations are performed by taking the anti-log of the mean of the log concentration transformations. Values to be used for the antibody concentrations below the assay cut-off will be described in the Statistical Analysis Plan (SAP).
  - Handling of missing data: for a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

- Reactogenicity and safety:
  - Handling of missing data: subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively). In case of significant non-compliance of study procedures for reporting symptoms, the analysis plan will be reassessed to ensure more accurate reporting of study data by further analysis.
  - For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore, the analysis of the solicited symptoms based on the All Enrolled Set will include only subjects/doses with documented safety data (i.e., symptom screen completed).

11.7. Analysis of demographics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment will be calculated overall and by vaccine/placebo group.

Distributions of subjects by gender and geographic ancestry will be summarized.

11.8. Analysis of efficacy

VE will be evaluated at the end of study period. VE will be estimated as 1-RR where RR is the risk ratio (proportion of subjects reporting the disease in the vaccinated group over the proportion in the placebo group) together with 90% CIs.

11.9. Analysis of immunogenicity

Analysis of binary variables:

The number and percentages of subjects will be summarized. Two-sided 95% CIs for the percentages will be computed.

Antibody titers/concentration below the limit of detection in the respective assay will be set to half the limit for the purposes of analysis. Missing values of immunogenicity will be excluded from analyses (i.e. complete-case analysis) since they are considered missing completely at random, i.e. not informative and with no impact on inferences.

Analysis of continuous variables:

The antibody concentrations/titers will be logarithmically transformed (base10) (to fulfil the normal distribution assumption). GMCs will be calculated, with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CI.

Additionally, within-subject geometric mean ratios (GMRs) will be computed for geometric mean titers/GMCs at each time point after vaccination versus baseline (Day 1). The GMRs and 95% CIs will be constructed by exponentiating the mean within-subject differences in log-transformed titers and the corresponding 95% CIs.

11.10. Analysis of safety

The primary analysis will be performed on the All Exposed Set.

Analysis of solicited local and systemic adverse events and other reactions:

Solicited AEs will be summarized according to defined severity grading scales.

Frequencies and percentages of subjects experiencing each AE will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic AE overall and at each timepoint will also be presented.

Post-vaccination/challenge solicited AE reported from Day 1 to 7 days post-vaccination/challenge will be summarized by maximal severity and by vaccine/placebo group. The severity of solicited local AE will be summarized according to categories based on linear measurement.

Injection-site pain and systemic reactions occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic AE will also be further summarized as “none” versus “any”.

Analysis of unsolicited AEs:

All the AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination/challenge by the investigator, will be recorded.

The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to preferred terms using the MedDRA dictionary. The AEs will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine/placebo or challenge agent, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine/placebo group will be counted.

Separate summaries will be produced for the following categories:

- SAEs.
- AEs that are possibly or probably related to vaccine/placebo or challenge agent.
- AEs leading to withdrawal from the study.

Data listings of all AEs will be provided by subject. In addition, AEs in the categories above will be provided as listed data.

11.11. Interpretation of analyses

Except for analyses on objectives with a predefined success criterion and an appropriate type I error control (see Section 2), comparative analyses will be descriptive with the aim to characterize the difference in reactogenicity and immunogenicity efficacy between groups. These descriptive analyses should not be interpreted.

11.12. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

11.12.1. Sequence of analyses

The total of 72 subjects will be divided in 4 cohorts of 18 subjects each (for bed availability at the study site). The vaccination and challenge will be done per cohort. However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.

The following 2 interim analyses are planned:

1. ***After completion of the Visit 5 (Day 64): post-challenge efficacy data of all subjects (all 4 cohorts)***
2. ***After Visit 6 (Day 85): secondary immunogenicity data and tertiary SBA immunogenicity data of all subjects (all 4 cohorts).***

(Amended: 23 July 2019)

All analyses will be conducted before unblinding on ***clean and final*** data. ***(Amended: 23 July 2019)***

An integrated ***C***linical ***S***tudy ***R***eport containing all data will be written ***after the End of Study*** and made available to the investigators. ***(Amended: 23 July 2019)***

If the data for tertiary endpoints become available at a later stage, (an) additional analysis/analyses will be performed. These data will be documented in annex(es) to the study report and will be made available to the investigators at that time.

11.12.2. Statistical considerations for interim analyses

No statistical adjustment will be made for the interim analyses, which are intended to provide final outputs related to the different endpoints and timepoints in a phased manner.

12. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, public disclosure requirements and publications must be fulfilled.

12.1. Electronic case report form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with an electronic format in read only mode of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

12.2. Subject Diary

Paper Diaries (pDiaries), hereafter referred to as Subject Diaries will be the only source document allowed for solicited local and systemic adverse events (including body temperature measurements), starting after the initial 30 minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the Subject Diary.

The Investigator or delegated staff should monitor the Subject's Diary status throughout the study for compliance and any solicited local and systemic adverse events that were of concern to the subject.

- No corrections or additions to the information recorded by the subject within the Subject Diary will be allowed after it is delivered to the site.
- Any blank or illegible fields on the Subject Diary must be described as missing in the eCRF.

The following additional rules apply to documentation of Subject Diary information collected in the eCRFs:

- The site must enter all readable entries in the Subject Diary into the eCRF, including those values that may be biologically implausible (e.g. body temperature: 400°C).

- Any illegible or implausible data should be reviewed with the Subject. If an underlying solicited or unsolicited AE is described on review with the subject, this should be described in the source document and reported as an unsolicited AE in the Adverse Event eCRF (e.g., if the subject above confirms body temperature of 40°C on the day in which body temperature: 400°C was written into his/her Subject Diary, this fever of 40°C should be recorded in the Adverse Event eCRF).
- Any newly described safety information (including a solicited AE) must not be written into the Subject Diary and must be described in the study file as a verbally reported AE. Any AE reported in this fashion must be described as an unsolicited AE and therefore entered on the Adverse Event eCRF.

12.3. Study monitoring by GSK biologicals

GSK will monitor the study to verify that, among other items, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

12.4. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures, otherwise, the minimum retention period will default to 25 years after completion of the study report.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

12.5. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

12.6. Posting of information on publicly available clinical trial registers and publication policy

GSK assures that the key design elements of this protocol will be posted on the GSK website and in publicly accessible database(s) such as clinicaltrials.gov, in compliance with the current regulations.

GSK also assures that results of this study will be posted on the GSK website and in publicly accessible regulatory registry(ies) within the required timeframe, in compliance with the current regulations. The minimal requirement is to have primary endpoint summary results disclosed at latest 12 months post PCD and to have secondary endpoint disclosed at latest 12 months after the Last Subject Last Visit as described in the protocol.

GSK also aims to publish the results of these studies in searchable, peer reviewed scientific literature and follows the guidance from the International Committee of Medical Journal Editors.

12.7. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

12.8. Data sharing

Under the framework of the SHARE initiative, results of GSK studies may be combined with non- GSK studies, to investigate further about the study product(s) and other product(s), and /or the disease/condition under investigation and related diseases and conditions.

12.9. Pharmacogenomics

As part of the sequencing for the microbiome, human genome will be sequenced as bypass product but the intent is not to analyze the data which will be stored securely.

Analyses of the transcriptomics signals evaluating the number of differential expressed genes by comparing the relative abundance of mRNA sequences are also considered a pharmacogenomics experiment.

By United States federal regulation, annual reports must include relevant pharmacogenomics results experiment. Refer to the US Guidance for industry “Pharmacogenomic data submissions” for more information on the process and format for submission of such data.

13. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

14. REFERENCES

Bodhidatta L, Pitisuttithum P, Chamnanchanant S, Chang K, Islam D, Bussaratid V, Venkatesan M, Hale T. Establishment of a *S. sonnei* human challenge model in Thailand. *Vaccine.* 2012 Nov; 30 (49): 7040-5.

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Cohen D, Block C, Green MS, Lowell G, Ofek I. Immunoglobulin M, A, and G antibody response to lipopolysaccharide O antigen in symptomatic and asymptomatic Shigella infections. *J Clin Microbiol*. 1989 Jan; 27 (1): 162–167.

Cohen D, Green M, Block C, Slepon R, Ambar R, Wasserman SS, Levine MM. Reduction of transmission of shigellosis by control of houseflies (*Musca domestica*). *Lancet*. 1991 Apr 27; 337 (8748): 993-7.

EMA Guideline on the exposure to medicinal products during pregnancy: need for post-authorization data (Doc. Ref. EMEA/CHMP/313666/2005) 'adopted at Community level in May 2006); *http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500011303.pdf*

Ferreccio C, Prado V, Ojeda A, Cayyazo M, Abrego P, Guers L, Levine MM. Epidemiologic patterns of acute diarrhea and endemic Shigella infections in children in a poor periurban setting in Santiago, Chile. *Am J Epidemiol*. 1991 Sep 15; 134 (6): 614-27.

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Gerke C, Colucci AM, Giannelli C, Sanzone S, Vitali CG, Sollai L, Rossi O, Martin LB, Auerbach J, Di Cioccio V, Saul A. Production of a *Shigella sonnei* Vaccine Based on Generalized Modules for Membrane Antigens (GMMA), 1790GAHB. *PLoS One*. 2015; 10 (8): e0134478.

Pitisuttithum P, Islam D, Chamnanchanunt S, Ruamsap N, Khantapura P, Kaewkungwal J, Kittitrakul C, Luvira V, Dhitavat J, Venkatesan MM, Mason CJ, Bodhidatta L. Clinical trial of an oral live *Shigella sonnei* vaccine candidate, WRSS1, in Thai adults. *Clin Vaccine Immunol*. 2016; 23: 564-575.

Appendix A LABORATORY ASSAYS

Hematology, Virology, Blood chemistry, HLA-B27, anti *S. sonnei* LPS IgG-Screening, urinalysis:

Hematology, Virology, Blood chemistry, HLA-B27, urinalysis and anti *S. sonnei* LPS IgG Screening-ELISA will be done at the local site according to the methods available there. The anti *S. Sonnei* LPS IgG Screening-ELISA will be done locally for the anti-*S. sonnei* LPS IgG antibody determination at screening.

HEMATOLOGY
White Blood Cells (WBC)
Red Blood Cells (RBC)
Hemoglobin
Hematocrit
Platelets
Eosinophils
Basophils
Neutrophils
Monocytes
Lymphocytes
CLINICAL CHEMISTRY
Total bilirubin
Aspartic Aminotransferase (ASAT/GOT)
Alanine Aminotransferase (ALAT/GPT)
γ-Glutamyl Transferase (γ-GT)
Lactic Dehydrogenase (LDH)
Alkaline Phosphatase (AP)
Total Proteins
Glucose (random glucose)
Blood Urea Nitrogen (BUN)
Creatinine
Sodium
Potassium
Prothrombin time (only at screening)
SEROLOGY for VIROLOGY
HBsAg
HCV antibodies
HIV antibodies
PREGNANCY TEST
Human chorionic gonadotropin (hCG) in urine
HLA-B27
HLA-B27
URINE DIPSTICK
Glucose
Proteins
pH
Ketones
Nitrites
Blood
URINALYSIS: Microscopic test on urine (performed if urine dipstick shows deviations from normal values)
Leucocytes (WBC)
Erythrocytes (RBC)
Epithelial Cells
Casts
Bacteria

Anti *S. sonnei* LPS antibody determination from serum:

Anti-*S. sonnei* LPS IgG antibodies in serum (for all timepoints except screening) will be measured by ELISA in the GVGH-Laboratories (Siena, Italy). The ELISA is performed using *S. sonnei* LPS as coating antigen. A standard curve is run in duplicate on each plate. Results are expressed in ELISA units (EU) /mL of serum. 1 unit equals the reciprocal of the dilution to give an OD of 1.

Serum Bactericidal Activity in serum:

Serum bactericidal activity against *S. sonnei* will be measured by serum bactericidal assay. This assay is currently in development in a GVGH laboratory.

S. sonnei determination from stool:

- *Culture:* The presence of S. *sonnei* in stool samples will be assessed using a qualitative procedure and following the SOPs of the local or designated laboratory for culture, isolation and identification of S. *sonnei* from stool specimens.
- *qPCR*: S. sonnei qPCR determination will be done following the SOPs of the local or designated laboratory for identification of *S. sonnei* by a qPCR from stool specimens.
- *S. sonnei* s*IgA* and total sIgA *determination from stool*: *S. Sonnei* ELISA will be performed following the SOPs of the local or designated laboratory for ELISA assay from stool specimens. The S. sonnei sIgA result will be normalized to the total sIgA.

Stool inflammatory markers assay

Inflammatory markers including calprotectin and myeloperoxidase levels in stools (ng/mL) will be measured using commercially available (Epitope Diagnostics, Inc.) quantitative ELISA kits according to the manufacturer's instructions.

α4β7 plasmablast ELISA (Amended: 23 July 2019)

Anti-S. sonnei LPS-specific IgG titers will be determined in plasmablasts, in both α4β7 positive and α4β7negative populations. The titers provided will be measured by ELISA assay in the culture supernatant of 5x10^6 cells, by using an in-house method developed in WRAIR. (Amended: 23 July 2019)

Transcriptomics

The transcriptomics signals analysis will be performed evaluating the number of differential expressed genes by comparing the relative abundance of mRNA sequences.

Gut Microbiome profile:

Gut microbiome analysis in stool using 16s ribosomal DNA amplicon sequencing and/or shotgun whole genome sequencing (refer to Section 6.7.3).

Glycosylation profiles of IgG antibodies:

The activity of IgG antibody is mediated through two functional domains, the Fab and the Fc. The Fc domain mediates effector functions though specific interactions with FcγRs expressed on the surface of leukocytes, and activation of diverse immunomodulatory pathways impacting both innate and adaptive immunity [1]. The mechanisms of Fc domain diversification that determine Fc-FcγR interaction include IgG subclass and Fc-associated glycan structure [2]. Distinct profiles are enriched following vaccination or during inflammatory diseases or infection. Vaccination with 1790GAHB is hypothesized to enrich certain IgG subclasses and Fc glycan structures and specific profiles of elicited IgG will contribute to clinical protection observed in controlled human infection models.

References for Appendix A:

1. Bournazos S and Ravetch JV. Fcγ receptor pathways during active and passive immunization. Immunol Rev. 2015; 268:88-103.
2. Pincetic A et al. Type I and type II Fc receptors regulate innate and adaptive immunity. Nat Immunol. 2014; 15:707-716.

Appendix B CLINICAL LABORATORIES

Table 22 GSK Biologicals' laboratories

Laboratory	Address
GSK Biological's Clinical Laboratory Sciences, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart – Belgium
GVGH Laboratory	Via Fiorentina 1 53100 Siena Italy

Table 23 Outsourced laboratories

Laboratory	Address
Cincinnati Children's Hospital Medical Center Core Laboratories	3333 Burnet Avenue, Building B, MLC 1010 Cincinnati, Ohio 45229-3039, USA
Cincinnati Children's Hospital Medical Center Clinical site Laboratories	619 Oak street, Room 2803 Cincinnati, Ohio, 45206, USA
Rockefeller University	1230 York Avenue New York, NY 10065, USA
WRAIR	503 Robert Grant Avenue Silver Spring, MD 20910

Appendix C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals SA Vaccines R&D **Protocol Amendment 1**	
eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 1
Amendment date:	08 February 2018
Coordinating author:	PPD (XPE Pharma for GSK Biologicals)
Rationale/background for changes: • To increase the yield of the peripheral blood mononuclear cell (PBMC) analysis, volume of the blood collected for PBMC analysis has been changed from 40 mL to 50 mL. • To make the definition of the primary objective more conclusive, the wording of the primary objective definition has been updated. • The case definition of shigellosis has been also reworded to make it complete and more conclusive.	

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Section 2.1 Primary objective (+ in synopsis)

- To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo in preventing (reduction of shigellosis, fulfilling according to the protocol primary case definition, i.e. moderate or severe diarrhea, OR oral temperature of ≥ 38°C after challenge with *S. sonnei* strain 53G).

Section 2.2 Secondary objective (+ in synopsis)

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - […]
  - Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery ~~AND~~ ***OR*** presence of fever ≥ 38°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, tenesmus, nausea, vomiting …).

Section 3 Study design overview (+ in synopsis)

- **Sampling schedule:**
  - […]
  - ~~40~~ ***50*** mL of peripheral venous blood for α4β7 plasmablast flow-cytometry and RNA extraction from PBMCs for gene expression profiling: Visit 1, Visit 2, Visit 4 and 7 days after Visit 5 (Day 64).

Section 3.2 Challenge phase

Four weeks after completion of the two-dose vaccination schedule (approximately 28 days after Visit 4 ***3***) subjects will be admitted the day before the challenge date […]

Section 4 Case definitions (+ in synopsis)

Shigellosis

For the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Moderate or severe diarrhea OR fever ***(oral temperature ≥ 38°C).***

Section 5.6 Outline of study procedures

Table 4 List of study procedures

Blood sampling for PBMC isolation (α4β7 plasmablasts response and transcriptomics) (~~40~~ ***50*** mL)

Section 6.6.10.1 Blood sampling for safety or immune response assessments

- An overall volume of 360 400 mL will be collected per subject during the entire study period (8 months).

Section 6.7.2 Biological samples

Table 6 Biological samples

Sample type (analysis) (All subjects)	Quantity	Unit	Timepoint
Blood (PBMC for α4β7 plasmablasts response and transcriptomics)	~ ~~40~~ ***50***	mL	scheduled

[…]
**Includes anti *S. sonnei* LPS IgG-ELISA (~~Clinical Laboratory Sciences~~)

Section 6.7.3 Laboratory assays

Table 10 Hematology, Serum Chemistry, Virology, HLA-B27 test, Urine and Stool tests

System	Component	Method	Laboratory
EDTA Blood	HLA-B27	~~Flow Cytometry~~ ***PCR***	Study site

[…], ***PCR: Polymerase Chain Reaction***

Section 6.7.4.1 Immunological read-outs

Table 11 Immunological read-outs

Note: Anti-*S. sonnei* LPS IgG will be determined by local Screening-ELISA at screening and by ~~Clinical Laboratory Sciences~~ ***GVGH*** ELISA for the rest of the timepoints.

Section 11.2 Secondary endpoints (+ in synopsis)

Efficacy

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured against:

- […]
- Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery ~~AND~~ ***OR*** presence of fever ≥ 38°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, tenesmus, nausea, vomiting …).

Section 12.9 Pharmacogenomics

As part of the sequencing ***for the microbiome***, human genome will be ~~sequence~~ ***sequenced*** as bypass product but the intent is not to analyze the data which will be store securely.

Analyses of the transcriptomics signals evaluating the number of differential expressed genes by comparing the relative abundance of mRNA sequences are also considered a pharmacogenomics experiment.

Appendix A Laboratory assays

Anti *S. sonnei* LPS antibody determination from serum:

Anti-*S. sonnei* LPS IgG antibodies in serum (for all timepoints except screening) will be measured by ELISA in the ~~Clinical Laboratory Sciences laboratories of GSK Biologicals~~ ***GVGH-Laboratories, Siena***. The ELISA is performed using *S. sonnei* LPS […]

Appendix B Clinical laboratories

Table 22 GSK Biologicals' laboratories

Laboratory	Address
~~GSK Vaccines GmbH~~ ~~Clinical Laboratory Sciences, Marburg, Germany~~	~~Emil-von-Behring-Str. 76~~ ~~35041 Marburg~~ ~~Germany~~

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 2

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 2
Amendment date:	22 June 2018
Coordinating author:	PPD

Rationale/background for changes:

- To address FDA comment that the case definition as stated in Protocol Amendment 1 did not appear to be sufficiently specific for shigellosis versus other causes of fever: therefore shedding has been added to address the deficiency in the primary case definition.
- The definition of fever in the previous case definition (Temperature ≥38°C) has been changed to (Temperature ≥38.5°C) to be in line with the definition of fever used for the challenge model set up at Cincinnati.
- To address FDA comment on performing screening safety laboratories but not proposing eligibility criteria based on them.
- To address the FDA request to provide a sample size calculation based on human challenge data relevant to the primary endpoint case definition in this study.
- To incorporate FDA recommendation on definition of holding rule for the study.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Section 2.1 Primary objective (+ in synopsis)

- To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo (Reduction of shigellosis according to the protocol primary case definition*, i.e.* ***Shedding of S. sonnei 53G accompanied by moderate or severe diarrhea,*** OR ***shedding with an*** oral temperature of ≥ 38***.5°C*** after challenge with *S. sonnei* strain 53G***)***.

Section 2.2 Secondary objective (+ in synopsis)

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - […]

– Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of fever ≥ 38**.5°**C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, tenesmus, nausea, vomiting …).

Section 4 Case definitions (+ in synopsis)

Shigellosis

For the challenge phase of the study, the protocol primary case definition for shigellosis is:

- ***Shedding of S. sonnei 53G accompanied by m***~~M~~oderate or severe diarrhea OR ***shedding with*** ~~fever (~~***an oral temperature of*** ≥ 38***.5°C***~~)~~.

Section 5.3 Exclusion criteria

Subjects with a baseline neutrophil count below 1800 cells/µL LLN ***OR with clinically significant abnormalities in other laboratory values (CBC, CMP, UA), according to local reference ranges and investigator judgment)***

Section 9.10.1 Holding rule

The following holding rule has been defined for the study: at least one subject developing a ≥Grade 3 symptomatic neutropenia considered possibly, probably or definitely related ~~within 7 days after~~ ***to*** vaccination

Section 11.2 Secondary endpoints

Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of fever ≥38***.5***°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, tenesmus, nausea, vomiting …).

Section 11.4 Determination of sample size

Based on the results obtained by the Cincinnati Children's Hospital Medical Center, where the challenge model was developed, in subjects receiving a challenge with 1500 CFU of S. sonnei 53G, the attack rate for the primary case definition of this study is 58%. Thus considering an AR of ~~60~~***58%*** in the placebo group and a percentage of non-evaluable subjects of ***22%***, approximately 72 subjects (36 per group) will be recruited to reach these 21 cases.

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 3

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 3
Amendment date:	07 September 2018
Coordinating author:	PPD

Rationale/background for changes:

- To address a comment made by the FDA asking if listings of unblinded unsolicited adverse events after challenge are made available to the IDMC.
- To add a secondary objective for evaluating efficacy of the vaccine also against a consensus definition of shigellosis by the *Controlled Human Infection Model (CHIM) expert working group* that was convened by the Gates Foundation, the funder of the study, on 06 February 2018.
- To clarify that regarding weight and number of stools, the interest is in total number and weight of all grade 3-5 stools, and not only in grade 3-5 stools concurring in diarrhea episodes.
- To further harmonize the protocol with the challenge model established at the Cincinnati Children's Hospital Medical Center with previous GVGH studies in terms of grading of events and immunological parameters evaluated, and with GSK standards.
- To introduce a mitigation strategy in case the site is not able to include the required number of subjects in a given cohort.
- To add a tertiary objective to evaluate inflammatory response in stools as discussed with the funder of the study and other partners.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover Page:

Investigational New Drug (IND) number	***IND18163***
Contributing authors	PPD, PPD, ***GVGH Serology Representatives (Scientific Technical Leaders)***

List of Abbreviations

CHIM:	*Controlled Human Infection Model*
ICH:	International ***Council for Harmonisation*** ~~Conference on Harmonization~~

Section 1.3.1 Risk Assessment

The most common symptoms that happen after taking ~~Bactrim~~ ***TMP-SMX*** are nausea, vomiting, loss of appetite, skin rash and itching.

Section 1.3.3 Overall Benefit: Risk Conclusion

However the *S. sonnei* 53G challenge strain used in the challenge phase is known to be susceptible to multiple antimicrobial agents including TMP-SMX and quinolones which will be used in this study and will be given to all challenged subjects ~~at Day 5 of the challenge phase (4th day after administration of the challenge agent)~~ ***5 days after administration of the challenge agent (day 62)*** or earlier if medically needed.

Section 2.2 Secondary objectives (+ in synopsis)

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - ***Shigellosis as defined by the CHIM expert working group on case definition.****
  - […]
  - Weight of ***all*** grade 3-5 ~~diarrhea episodes~~ ***stools***.
  - Total number of ***all*** grade 3-5 ~~diarrhea episodes~~ ***stools***.
  - Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of ***oral temperature*** ~~fever~~ ≥ 38.5°C OR presence of one or more severe intestinal symptoms ***(abdominal pain, cramping,*** ~~tenesmus,~~ ***nausea, vomiting~~...~~, gas, and anorexia).***
  - […]
  - ***Time to onset of shigellosis after challenge, according to the primary case definition.***

****According to the working group, the participant must fulfil any one of the three following possible endpoints to qualify as having reached the CHIM case definition for this objective:***

1. ***Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]***
2. ***Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0oC OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]***

3. ***Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0oC OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].***

Safety

- To assess the safety and reactogenicity of the 1790GAHB vaccine in terms of solicited ~~symptoms~~***adverse events***, unsolicited ~~symptoms~~***adverse events***, serious adverse events (SAEs), adverse events of special interest (AESI) and laboratory parameters.
- To assess safety after challenge in terms of SAEs, unsolicited adverse events, AESI, and laboratory parameters.

Immunogenicity

- To evaluate the IgG ELISA immunogenicity profile of the 1790GAHB vaccine at ***7 days and*** 28 days after the first and second vaccination (IgG ELISA coated with OAg containing LPS).
- […]
- ***To assess anti- S.sonnei LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.***

Section 2.3 Tertiary objectives (+ in synopsis)

- […]
- ***Evaluate inflammatory response from stools collected before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64)***
- […]

Section 3 Study Design Overview

The total of 72 subjects will be divided in 4 cohorts of 18 subjects each (for logistical reasons due to bed capacity at the study site). The vaccination and challenge will be done in overlapping cohorts and randomization will ensure there is the same number of vaccinees and controls in each cohort. ***However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.***

Section 3.2 Challenge Phase

- **Sampling schedule:**
  - […]
  - ***Stool sample for inflammatory response before challenge at Visit 5 (Day 57) and daily, post challenge, during inpatient stay (Day 57 to Day 64)***
  - […]

- **Safety monitoring:**
- […]

The safety review team (SRT) will monitor the safety of subjects throughout the study. The SRT will review blinded data, assess safety signals and make recommendations to the ***IDMC and*** Sponsor concerning continuation, termination, or other modifications of the study based on the observed adverse effects.

Section 4.1 Diarrhea (+ in synopsis)

For the purpose of this study, diarrhea is defined as:

- […]
- Severe diarrhea: 6 or more loose or watery (Grade 3 to 5) stools or > 800 grams of Grades 3 to 5 stools within 24 hours or required medical intervention. ***In case of severe diarrhea, medical intervention is defined as intravenous (IV) fluids administration or anticipation of antibiotic treatment before the 5th day after challenge.***
- More severe diarrhea: ~~≥ 10 loose~~ ***10 or more loose or watery*** (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours or required medical intervention. ***In case of more severe diarrhea, medical intervention is defined as ER visit or hospitalization for hypotensive shock.***

Section 4.3 Shigellosis (+ in synopsis)

For the ***primary and secondary objectives in the*** challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea OR shedding with *an oral temperature of ≥ 38.5°C).*

For the secondary objectives in the challenge phase of the study, the CHIM working group case definition will require the participant to fulfil any one of the three following possible endpoints:

1. ***Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]***
2. ***Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]***
3. ***Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0^{o}C0 °C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].***

Section 5.1 Number of subjects/center

Enrolment will take place in 4 overlapping cohorts of 18 subjects each to accommodate for the availability of beds required for the challenge phase of the study at the clinical

site (see also Section 11.12.1). However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort. The vaccination and challenge will be done per cohort.

Section 6.2.2.2.1 Study group and treatment allocation

Subjects will be enrolled in 4 overlapping cohorts of 18 subjects each, for logistical reasons at the clinical site, therefore a 1:1 ratio should be maintained at the cohort level. ***However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.***

Table 4 List of Study Procedures

Record any concomitant medications/vaccinations	● ***Visit 8***
Stool sample for inflammatory response	● ***Visit 5,*** ● ***Inpatient Stay***
Recording of solicited adverse events ***within 7 days post-vaccination***	
Recording of ~~non-serious~~ ***unsolicited*** adverse events within 28 days post-vaccination	
Recording of solicited adverse events within 7 days post-challenge	● ***Inpatient Stay***
Recording of unsolicited adverse events within 28 days post-challenge	●***Inpatient Stay*** ●***Visit 6***
Recording of AESI, SAEs and pregnancies ~~●***Phone call (Day 3 and Day 7)*** ●***Phone call (Day 31 and Day 35)***~~	
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	~~●***Phone call (Day 3 and Day 7)*** ●***Phone call (Day 31 and Day 35)***~~

[7] Before vaccination/challenge (Day 1/Day 57 sample can be collected by the subject at most a week before and stored ***as described in the Investigator Manual and SPM.***

Table 5 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Visit 5 → Visit 7	56 days	~~56~~***49 days*** - ~~70~~***63 days***
Visit 5 → Visit 8	180 days	~~180~~***166 days*** - 194 days

Section 6.6.8 Assess pre-vaccination body temperature

The oral body temperature of each subject needs to be measured prior to any study vaccine/placebo or challenge agent administration. If the subject has fever (fever is defined as temperature ≥ 38.0°C/100.4°F ~~regardless the location of measurement~~) on the day of vaccination, the visit will be rescheduled within the allowed interval (see Table 5).

Section 6.6.10.3 Stool sampling

Aliquots of stool will be collected for fecal sIgA, inflammatory response, culture and qPCR, at each predefined timepoint (see Table 4). If, during the inpatient stay, no stools are passed on a given day, up to two rectal swabs can be collected for culture and qPCR (see also Section 3.2). The aliquots for fecal sIgA and inflammatory markers should be frozen at -80°C as soon as possible at the clinical site.

Section 6.6.12 Challenge administration and inpatient stay

Refer to Section 3.2 and SPM for more details related to ~~challenge administration and~~ ***the preparation and administration of the challenge agent and the collection of samples during the*** inpatient stay.

Section 6.6.14.1 Subject Diary

Subject Diary training should be directed at the individual(s) who will perform the measurements of adverse events and who will enter the information into the Subject Diary. This individual may not be the subject, but if a person other than the subject enters information into the Subject Diary, this person's identity must be documented in the ~~Subject Diary~~ ***Subject's source records***. Any individual that makes entries into the Subject Diary must receive training on completion of the Subject Diary at the time of the visit when Subject Diary is dispensed. This training must be documented in the subject's source record.

Section 6.7 Biological sample handling and analysis

Please refer to the SPM ***and Investigator Manual*** for details on biospecimen management (handling, storage and shipment).

Table 6 Biological samples

Stool*** (sIgA, microbiome, ***inflammatory markers,*** weight, consistency and blood assessment, culture and qPCR)			scheduled

Table 10 Hematology, Serum Chemistry, Virology, HLA-B27 test, Urine and Stool tests

Stool	***Inflammatory markers***	***ELISA***	***WRAIR or GVGH designated lab***

ELISA: Enzyme-Linked Immunosorbent Assay, WRAIR: Walter Reed Army Institute of Research (US)

Section 6.7.3 Laboratory assays

- […]

Fecal markers of intestinal inflammation will be measured as a sensitive predictor for inflammatory activities in the gastrointestinal tract. This analysis will assess if concentrations of inflammatory markers such as calprotectin and myeloperoxidase will increase after oral challenge with S. sonnei 53G and will additionally investigate if the S. sonnei 1790GAHB vaccine can reduce or abrogate the inflammatory response in the intestine after oral challenge.

Table 12 Hematology/Blood Chemistry read-outs

Visit 8 (Day ~~240~~ ***237)***	Post-challenge	72	Hematology

Section 7.6.1 Recording of concomitant medications/products and concomitant vaccinations

- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

E.g., an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring (fever is defined as temperature ≥ 38.0°C/100.4°F ~~regardless the location of measurement~~). The preferred location for measuring temperature in this study will be the oral cavity.

Section 9.1.3 Solicited adverse events

~~The following solicited AEs~~***Solicited AEs after vaccination*** are included in the Subject Diary. Each AE is to be assessed using the scoring system reported in ~~parentheses below~~***Section 9.3.3.2.1.***

Section 9.1.3.3 Solicited events after challenge phase

The hydration of subjects developing diarrhea will be maintained with oral electrolyte solutions ~~(i.e., Pedialyte, Gatorade)~~by offering at least 1.5 mL for each gram of diarrheal stool lost, as tolerated.

Section 9.1.4 Unsolicited adverse events

Unsolicited AEs will be collected until 28 days after each vaccination *and until 28 days after challenge*.

Table 19 Reporting periods for collecting safety information

Timepoints	Scr	D1	D8	D29	D36	D57	D57-D64	D85	D237 (M8)
Solicited local and systemic AEs									
Unsolicited AEs									

Table 20 Intensity scales for solicited symptoms

Pain at injection site	0	~~None~~***Absent***
	1	~~Mild: Any pain neither interfering with nor preventing normal every day activities.~~ ***Easily tolerated.***
	2	~~Moderate: Painful when limb is moved and interferes with every day activities~~ ***Interferes with normal activity***
	3	~~Severe: Significant pain at rest. Prevents normal every day activities.~~***Prevents normal activity.***
Headache#	0	~~*Normal*~~***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
Fatigue#	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
Arthralgia#	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
Malaise#	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
Myalgia#	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
Chills	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	~~***That***~~ *Prevents* normal activity
After challenge agent administration		
Nausea	0	~~*Normal*~~***Absent***
	1	Mild or transient; maintains reasonable intake
	2	Moderate discomfort; intake decreased significantly; some activity limited
	3	No significant intake and requires medical intervention
	4	Hospitalization required
Abdominal cramping	0	~~*Normal*~~***Absent***
	1	No interference with daily activities
	2	Some interference with daily activities not requiring medical intervention
	3	Prevents daily activities and requires medical intervention
	4	ER visit or hospitalization
Abdominal pain	0	***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Gas	0	~~*Normal*~~***Absent***
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Anorexia	0	~~*Normal*~~***Absent***
	1	Easily tolerated
	2	Interferes with normal activity

	3	Prevents normal activity
Vomiting	0	~~*Normal*~~***Absent***
	1	One episode within a 24-hour period Vomiting
	2	2 episodes within a 24-hour period
	3	>2 episodes within a 24-hour period and requires medical intervention
	4	ER visit or hospitalization for hypotensive shock
Diarrhea**	0	~~*Normal*~~***Absent***
	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools (loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours ~~and~~ ***or*** requires medical intervention
	4	≥ 10 loose stools (Grade 3 to 5) or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours or ER visit or hospitalization for hypotensive shock

Section 9.3.3.2.1 Assessment of intensity

	Erythema/Induration
0:	~~≤ 20~~ **< *25*** mm
1:	~~> 20~~ **≥ *25*** - ≤ 50 mm
2:	> 50 - ≤ 100 mm
3:	> 100 mm

Section 9.10.2 Safety monitoring

The SRT will monitor the safety of subjects throughout the study. The SRT will review blinded data, assess safety signals and make recommendations ***to the IDMC and to the Sponsor*** concerning continuation, termination, or other modifications of the study based on the observed adverse effects.

Section 11.1 Primary endpoint (plus Synopsis)

- Rate of shigellosis (fulfilling the protocol ***primary*** case definition) occurring within a period starting with the challenge visit and lasting up to the end of the inpatient stay, in all subjects.

Section 11.2 Secondary endpoints (plus Synopsis)

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured ***during inpatient stay (Day 57 to Day 64)*** against:

- ***Rate of shigellosis (fulfilling the CHIM working group case definition for shigellosis) occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects.***
- […]
- Weight of ***all*** grade 3-5 ~~diarrhea episodes~~***stools.***
- Total number of ***all*** grade 3-5 ~~diarrhea episodes~~***stools***.

- Confirmed *S. sonnei* 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature fever ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, tenesmus, nausea, vomiting, …gas, anorexia).
- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- ***Time to onset of shigellosis after challenge, according to the protocol primary case definition.***

Safety

Solicited adverse events

- Occurrence of each solicited local and systemic adverse event within 7 days after each vaccination.

Unsolicited adverse events

- Occurrence of unsolicited AEs within 28 days after ~~*any*~~ vaccination ***and after challenge***, according to Medical Dictionary for Regulatory Activities (MedDRA) classification.
- […]

Immunogenicity

The measures of immunogenicity, against the LPS of *S. sonnei*, will include:

- IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), ***7 and*** 28 days after first and ~~28 days after~~ ***second vaccination*** by antibody concentration at baseline (i.e., above vs. below the assay detection limit), as determined by anti-*S. sonnei* LPS IgG ELISA.
- […]
- ***Number and percentage of subjects achieving a post vaccination anti- S. Sonnei LPS concentration ≥ 121 EU/ml at 28 days after first and second vaccination.***
- Number and percentage of seroresponders***** for anti-*S. sonnei* LPS at 28 days after first and second vaccination.

*****Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. […]

Section 11.3 Tertiary endpoints (plus Synopsis)

- […]
- Glycosylation profiles of IgG antibodies at Day 1 and 28 days after second vaccination (***Visit 5;*** Day 57).
- […]
- ***Concentration of stool markers of intestinal inflammation (e.g. calprotectin and myeloperoxidase) before oral challenge with S. sonnei, 53G at Visit 5 (Day 57) and daily during the post-challenge inpatient stay (Day 57 to Day 64)***
- […]
- Diversity and frequency of microbiome components (taxa and/or genes according to technology) at Day 1 prior to vaccination and 28 days after second vaccination (***Visit 5;*** Day 57)***.***

Section 11.5.7 Sub-groups

~~There are no intended sub-groups for analysis in this study.~~***The analysis for the primary efficacy endpoint will be repeated for the two subgroups of subjects who were responders or non-responders at the immunogenicity assessment of the pre-challenge visit.***

Section 11.12.1 Sequence of analyses

The total of 72 subjects will be divided in 4 cohorts of 18 subjects each (for bed availability at the study site). The vaccination and challenge will be done per cohort. ***However, if for any reason, a cohort does not reach the planned 18 subjects, additional subjects can be added in the next cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds in the clinic) in each cohort.***

One interim analysis is intended and will include post-challenge efficacy data of all subjects (all 4 cohorts) ~~and immunogenicity data up to 28 days after second vaccination for the first 3 vaccinated cohorts. Further serological results will follow after the conclusion of the study.~~.

Appendix A Laboratory Assays

[…]

Stool inflammatory markers assay

Inflammatory markers including calprotectin and myeloperoxidase levels in stools (ng/mL) will be measured using commercially available (Epitope Diagnostics, Inc.) quantitative ELISA kits according to the manufacturer's instructions.

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 4

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 4
Amendment date:	19 February 2019
Coordinating author:	PPD (XPE Pharma for GSK Biologicals)

Rationale/background for changes:

- Subjects of African descent are known to have a lower Absolute Neutrophil Count (ANC) than subjects of European descent. African Americans represent about 75% of all included subjects in the study so far, which is more than was initially anticipated. Furthermore, all cases of neutropenia recorded so far in the study occurred in African-American subjects. The threshold and grading scale, appropriate for this population, in line with the recommended criteria of the Division of AIDS (DAIDS), have been added to the protocol. This change will provide a more appropriate definition of neutropenia for African-American subjects as a basis to decide on eligibility for participation in the study and for vaccination and administration of the challenge agent, and will mitigate the risk that African-American subjects are excluded from the study due to episodes of neutropenia that are not clinically relevant.
- The current definition of "more severe diarrhea" was inconsistent with the definition of "Shigellosis". A change has been introduced to the definition of "more severe diarrhea" for a clear classification of cases.
- Minor editorial mistakes are corrected.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Contributing Authors

- PPD**, *Safety Scientist, Safety Evaluation and Risk Management team***

List of Abbreviations

DAIDS: ***Division of Acquired Immunodeficiency Syndrome***

Section 1.3.1 Risk Assessment

Important Potential/Identified Risk	Data/Rationale for Risk	Mitigation Strategy
Investigational vaccine: 1790GAHB vaccine		
Symptomatic Neutropenia	Eight cases of Grade 2 or Grade 3 neutropenia were reported from Phase 1 clinical trials (asymptomatic). All cases occurred in the first 20 days after administration of first vaccine dose, and reversed after discontinuation. Literature review of reports of neutropenia in association with vaccination indicates factors noted to be associated with neutropenia (including ethnicity, and low baseline counts) with the majority of cases detected within 2 weeks of the vaccination. None of the reported cases was clinically symptomatic. Five cases of asymptomatic neutropenia (Grade 1 and 2) have been reported at Day 7 after receipt of the first dose in 5 subjects. One of the 5 subjects had an additional Grade1 neutropenia episode at Day 28, and another one had two additional episodes, one of which was Grade 3 (940 cells/mL) at 7 days after the second dose and the second was Grade 2 (1,410 cells/mL) at 28 days after second vaccination. All cases were resolved before last study visit.	Exclusion criteria for this clinical ~~studies~~***study*** include: • Subjects with a low baseline neutrophil (below ~~1800 cells/µl LLN~~ ***1000 cells/µL for African-American subjects or other subjects of African descent and 1800 cells/µL for subjects of other ethnicity)***. As guidance, the ~~WHO criteria should be followed~~ ***Division of Acquired Immunodeficiency Syndrome (DAIDS) criteria [DAIDS, 2017] should be followed for African-American subjects or other subjects of African descent, whereas WHO criteria should be followed for all other subjects.*** • Previous history of Benign Ethnic Neutropenia, or drug related Neutropenia. • Concomitant treatment with neutropenic agents. Monitoring for this clinical study to include: • Baseline neutrophil counts. • Full blood counts with differential measured at 7 days post each scheduled vaccination. • Assessment of clinical impact of neutropenia. Subjects will be closely monitored during the entire study and have hematology repeated every 7 days when neutropenia is identified until resolution. If a case of neutropenia does not resolve ***based on most recent available laboratory results,*** before the second vaccination, ***or challenge agent administration,*** the subject will not be re-vaccinated ***or administered the challenge agent.***

Section 4.1 (+Synopsis) Diarrhea

For the purpose of this study, diarrhea is defined as:

- […]
- More severe diarrhea: 10 or more loose or watery (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours or required medical intervention. In case of more severe diarrhea, medical intervention is defined as ER visit or hospitalization for hypotensive shock.

Section 5.3 Exclusion criteria

- […]
- Subjects with a baseline neutrophil below ~~1800~~ ***1000*** cells/µL ~~LLN~~ ***for African-American subjects or other subjects of African descent, and 1800 cells/µL for subjects of other ethnicity*** OR with clinically significant abnormalities in other laboratory values (CBC, CMP, UA), according to local reference ranges and investigator judgment).

Section 7.5 Contraindications to subsequent vaccination or challenge

- […]
- ~~Neutropenia below 1800 cells/µL LLN.~~
- […]

The following event***s*** constitute~~s~~ contraindications to administration of 1790GAHB vaccine ***or the challenge agent*** at that point in time; if ~~this~~ ***any of these*** event***s*** occur~~s~~ at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 6.5), or the subject may be withdrawn at the discretion of the investigator (see Section 9.5):

- ***Neutropenia (for threshold values refer to Section 9.1.6) based on most recent available laboratory results. Subjects with previous neutropenia or unresolved neutropenia may be re-evaluated prior to vaccination or prior to challenge at the discretion of the investigator.***
- […]

Section 9.1.6 Adverse events of special interest

- […]

Neutropenia is defined as ***a*** decrease of neutrophil count asymptomatically or symptomatically. ~~Available local laboratory ranges will be used to define neutropenia.~~ This is completely diagnosed by laboratory testing for complete blood count. However, only symptomatic neutropenia cases will be considered as AESI and reported as such.

The following grading will be used to classify neutropenia in African-American subjects or other subjects of African descent:

- ***Grade 1: 1000-800 cells/µL.***
- ***Grade 2: 799-600 cells/µL.***
- ***Grade 3: 599-400 cells/µL.***
- ***Grade 4: < 400 cells/µL.***

The following grading will be used to classify neutropenia for subjects of other ethnicity:

- Grade 1: 1800-1500 cells/μL.
- Grade 2: 1499-1000 cells/μL.
- Grade 3: 999-500 cells/μL.
- Grade 4: < 500 cells/μL.

Section 9.3.3.2.1, Table 20 Intensity scales for solicited symptoms

Diarrhea**	0	Absent
	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools (loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours or requires medical intervention
	4	≥ 10 loose stools (Grade 3 to 5) or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours~~or ER visit or hospitalization for hypotensive shock~~

References

U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. (July 2017).
https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 5

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 5
Amendment date:	15 April 2019
Coordinating author:	PPD (XPE Pharma for GSK Biologicals)

Rationale/background for changes:

- To meet a FDA request for removing the DAIDS thresholds and criteria specific for African-American subjects that had been added in Amendment 4.
- To add secondary endpoints and case definitions for shigellosis that would facilitate comparison of results with other CHIM studies.
- To specify that stool samples will be analyzed for both *S. sonnei* secretory IgA (sIgA) and total sIgA (*S. sonnei* sIgA to be normalized for total sIgA).
- Minor editorial mistakes are corrected.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

List of Abbreviations

~~**DAIDS:** Division of Acquired Immunodeficiency Syndrome~~

Section 1.3.1. Risk Assessment (in the column "Mitigation Strategy)

Exclusion criteria for this clinical study include**:**

- Subjects with a ***low*** baseline neutrophil ***count*** below ~~1000~~***1800*** cells/µL ***(LLN)*** ~~for African-American subjects or other subjects of African descent and 1800 cells/µL for subjects of other ethnicity). As guidance, the Division of Acquired Immunodeficiency Syndrome (DAIDS) criteria [DAIDS, 2017] should be followed for African-American subjects or other subjects of African descent, whereas WHO criteria should be followed for all other subjects.~~

Section 2.2 Secondary objectives (+ in synopsis)

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - Shigellosis as defined by the CHIM expert working group on case definition.*

- ***Shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].***
- ***More severe shigellosis, as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 severe constitutional/enteric symptom]].***
- Shedding of *S. sonnei* strain 53G.
- […]

Section 2.3 Tertiary objective (+ in synopsis)

The following tertiary objectives which are part of the study exploratory objectives may be evaluated in addition to the primary and secondary objectives and will complement assessment of the vaccine immunogenicity profile:

- Evaluate ***S. sonnei specific secretory immunoglobulin A (***sIgA***)*** in stool at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- […]

Section 3 Study design overview (+ in synopsis)

- **Sampling schedule:**
  - […]
  - Stool sample: for microbiome testing at Visit 1 and Visit 5, for ***S. sonnei specific*** sIgA at Visit 1, Visit 2, Visit 4 and 7 days after challenge (Day 64) and stool analysis, culture and qPCR daily during inpatient stay (from Visit 5 to 7 days after challenge [Day 57 to Day 64] or prolonged until 2 consecutive negative stool samples if subject still shedding).

Section 4.2 Dystentery (+ in synopsis)

For the purpose of this study, dysentery is defined as:

- A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours and presence of constitutional***/enteric*** symptoms (e.g., fever, vomiting, chills, fatigue…***, refer to Table 23***).

Section 4.3 Shigellosis (+ in synopsis)

For the primary and secondary objectives in the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S. sonnei* 53G accompanied by moderate or severe diarrhea OR shedding with an oral temperature of ≥ 38.5°C).

For the secondary objectives in the challenge phase of the study, ***2 definitions of shigellosis are used:***

1. ***CHIM working group case definition for shigellosis as defined by any one of the three following possible endpoints:***
    - ***Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]***
    - ***Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]***
    - ***Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].***
2. ***Shigellosis as defined by any one of the three following possible endpoints:***
    - ***Severe diarrhea defined as [≥6 loose stools in 24 hours OR >800 grams loose stools in 24 hours]***
    - ***Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom].***
    - ***Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom].***

For the secondary objectives in the challenge phase of the study, more severe shigellosis is defined by any one of the two following possible endpoints:

- ***Moderate OR severe diarrhea [≥4 loose stools in 24 hours OR ≥400 grams loose stools in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom].***
- ***Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 severe constitutional/enteric symptom].***

~~the CHIM working group case definition will require the participant to fulfil any one of the three following possible endpoints:~~

~~Severe diarrhea defined as [≥6 loose stools in 24 hours] OR [>800 grams loose stools in 24 hours]~~

~~Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]~~

~~Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C0 °C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours].~~

Section 5.3 Exclusion criteria

- […]
- Subjects with a baseline neutrophil ***count*** below ~~1000 cells/µL for African-American subjects or other subjects of African descent, and~~ 1800 cells/µL ***(LLN)*** ~~for subjects of other ethnicity~~ OR with clinically significant abnormalities in other laboratory values (CBC, CMP, UA), according to local reference ranges and investigator judgment).
- […]

Section 6.7.3, Table 7 Immunity (Antibody determination)

System	Component	Method	Kit/ Manufacturer	Unit[2]	Cut-off[2]	Laboratory[3]
Serum (screening)	Anti-*S. sonnei* LPS IgG	ELISA[1]	WRAIR	Endpoint titer	plate dependent	CCHMC
Serum (except screening)	Anti-*S. sonnei* LPS IgG	ELISA	In-house	EU/mL	plate dependent	GVGH[4] or GVGH designated lab
Serum	Bactericidal antibodies	SBA	In-house	Dilution Factor (titer)	NA	GVGH[4] or GVGH designated lab
Stool	Anti-*S. sonnei* LPS ***s***IgA	ELISA	WRAIR	~~Titer~~ ***EU/mL***	***10*** ~~0.200 OD~~	CCHMC ~~or GVGH designated lab~~
Stool	***Anti-total sIgA***	***ELISA***	***WRAIR***	***mg/mL***	***0.001***	***CCHMC***

[1] Local Screening-ELISA will be used for this testing.
[2] Assay cut-off and unit might be subject to change during the course of the study (e.g. in case of requalification, revalidation or standardization). In this case, this will be documented in the clinical report.
[3] Refer to Appendix B for the laboratory addresses.
[4] GVGH laboratory refers to the GVGH-laboratories in Siena, Italy.
ELISA: enzyme-linked immunosorbent assay, **NA**: not applicable, **EU**: ELISA unit, **LPS**: lipopolysaccharide, **IgA/G**: immunoglobulin A/G, **SBA**: serum bactericidal Assay, **WRAIR**: Walter Reed Army Institute of Research (US), **GVGH**: GSK Vaccines Institute for Global Health, **CCHMC**: Cincinnati Children's Hospital Medical Center; ***sIgA: secretory Immunnoglobulin A***

Section 7.5 Contraindications to subsequent vaccination or challenge

~~[…]~~

The following event***s*** constitute contraindications to administration of 1790GAHB vaccine or the challenge agent at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 6.5), or the subject may be withdrawn at the discretion of the investigator (see Section 9.5):

- Neutropenia ~~(for threshold values refer to Section 9.1.6)~~***below 1800 cell/µL*** based on most recent available laboratory results. Subjects with previous neutropenia or unresolved neutropenia may be re-evaluated prior to vaccination or prior to challenge at the discretion of the investigator.
- […]

Section 9.1.6 Adverse events of special interest

~~[…]~~

The following grading will be used to classify neutropenia: ~~in African-American subjects or other subjects of African descent:~~

~~Grade 1: 1000-800 cells/µL.~~

~~Grade 2: 799-600 cells/µL.~~

~~Grade 3: 599-400 cells/µL.~~

~~Grade 4: < 400 cells/µL.~~

~~The following grading will be used to classify neutropenia for subjects of other ethnicity~~:

- Grade 1: 1800 -1500 cells/µL.
- Grade 2: 1499-1000 cells/µL.
- Grade 3: 999-500 cells/µL.
- Grade 4: < 500 cells/µL.

[…]

Section 11.2 Secondary endpoints (+ in Synopsis)

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured during inpatient stay (Day 57 to Day 64) against:

- Rate of shigellosis (fulfilling the CHIM working group case definition for shigellosis) occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects.
- ***Rate of shigellosis, as defined by severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 reportable constitutional/enteric symptom]].***
- ***Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 severe constitutional/enteric symptom]].***
- […]

Section 11.3 Tertiary endpoints (+ in Synopsis)

- Specific anti-S. sonnei LPS ***s***IgA antibody concentration in stool samples at Day 1, 7 days after first and second vaccination and 7 days after challenge (Day 64).
- […]

Section 14 References

~~U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. (July 2017).~~
https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

Appendix A LABORATORY ASSAYS

[…]

S. sonnei determination from stool:

- *Culture:* The presence of S. *sonnei* in stool samples will be assessed using a qualitative procedure and following the SOPs of the local or designated laboratory for culture, isolation and identification of S. *sonnei* from stool specimens.
- *qPCR*: S. sonnei qPCR determination will be done following the SOPs of the local or designated laboratory for identification of *S. sonnei* by a qPCR from stool specimens.
- *S. sonnei sIgA* ***and total sIgA*** *determination from stool*: *S. Sonnei* ELISA will be performed following the SOPs of the local or designated laboratory for ELISA assay from stool specimens. ***The S. sonnei sIgA result will be normalized to the total sIgA.***

GlaxoSmithKline Biologicals SA

Vaccines R&D

Protocol Amendment 6

eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Amendment number:	Amendment 6
Amendment date:	23 July 2019
Coordinating author:	PPD (Modis for GSK Biologicals)

Rationale/background for changes:

- To add an additional interim analysis for immunogenicity data, which will allow a faster interpretation of study results and planning of subsequent follow-up studies.
- To correct an error in the definitions of dysentery and more severe shigellosis, and align these definitions to other CHIM studies; oral temperature ≥ 38.0°C (fever) is added to the definitions.
- To correct an error in the primary completion date of the study.
- To clarify the different applicable case definitions of dysentery in the study.
- To provide clarification in the study design by showing the inpatient stay in the study design figure as per the Schedule of Activities table.
- To specify the allowed time interval window for the blood draw that occurs during the inpatient stay.
- To specify the assay used for the α4β7 plasmablast analysis as measuring both positive and negative cells by ELISA, and not using flow cytometry.
- To change the assay used for HLA-B27 testing from PCR to qualitative flow cytometry.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

List of Abbreviations

~~**ELISPOT:** Enzyme-linked immunospot assay~~

Section 2.2 Secondary objectives (+Synopsis)

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - […]
  - More severe shigellosis, as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptoms OR dysentery [[≥2

loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom]].

- […]

Section 2.3 Tertiary objectives (+Synopsis)

The following tertiary objectives which are part of the study exploratory objectives may be evaluated in addition to the primary and secondary objectives and will complement assessment of the vaccine immunogenicity profile:

- […]
- Evaluate ***S. Sonnei LPS IgG-specific*** ~~presence and migration of~~ α4β7***+/-*** plasmablast ***response*** at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- […]

Section 3.2, Figure 1 Study design

[…] **[…]**

Inpatient stay*
Day 57 to Day 65

B & S

****Refer to Section 3.2 for details of procedures during the inpatient stay***

Section 3.2 Challenge phase

[…]

Study Design

- **Primary completion date (PCD):** ~~Visit 8~~ ***End of inpatient stay (Visit 5,*** ~~6 months after challenge~~***Day 64)***.

[…]

- **Sampling schedule:**
  - […]
  - 50 mL of peripheral venous blood for α4β7***+/-*** plasmablast ~~flow cytometry~~***analysis by ELISA*** and RNA extraction from PBMCs for gene expression profiling: Visit 1, Visit 2, Visit 4 and 7 days after Visit 5 (Day 64).
  - […]

Section 4.2 Dysentery (+Synopsis)

~~For the purpose of this study, dysentery is defined :~~

~~A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours and presence of constitutional/enteric symptoms (e.g., fever, vomiting, chills, fatigue…, refer to 23).~~***For the purpose of the endpoint "Rate of shigellosis as defined by the CHIM***

expert working group occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]. In this case constitutional/enteric symptom are the following: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise.***

For the purpose of the endpoint "Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [≥1 reportable constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoint "Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]].", dysentery is defined as follows:

- ***At least 2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.***

For the purpose of the endpoints "Dysentery" and "Confirmed S. sonnei 53G shedding AND moderate or severe diarrhea OR dysentery OR presence of oral temperature ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia)", dysentery is defined as follows:

- ***A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours and presence of constitutional/enteric symptoms. In this case, constitutional/enteric symptoms consist of headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting, and fever.***

Section 4.3 Shigellosis (+Synopsis)

For the secondary objectives in the challenge phase of the study, more severe shigellosis is defined by any one of the two following possible endpoints:

- […]

– Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom].

Section 6.5, Table 4 List of study procedures

Blood sampling for PBMC isolation (α4β7***+/-*** plasmablasts response and transcriptomics) (~50 mL)

Section 6.5 Table 5 Intervals between study visits

Visit 5 → Visit 5 post-challenge blood draw	***7 days***	***7 days – 10 days***

Section 6.7.2, Table 6 Biological samples

Blood (PBMC) for α4β7***+/-*** plasmablasts response and transcriptomics)	~ 50	ml	scheduled

Section 6.7.3, Table 8 Cell-Mediated Immunity (CMI) ~~using ELISPOT~~

System	Component	Method	Unit	Laboratory
PBMC	α4β7 ***positive*** plasmablast	~~ELISPOT~~***ELISA***	***Anti- S. sonnei LPS specific Titer/ 5x10^6 cells***~~spot-forming units~~	WRAIR or GVGH designated lab
PBMC	***α4β7 negative plasmablast***	***ELISA***	***Anti- S. sonnei LPS specific Titer/ 5x10^6 cells***	***WRAIR or GVGH designated lab***
PBMC	Gene expression (transcriptomics) study	NextGen sequencing/ microarray	Sequencing or fluorescent intensity	GVGH* or GVGH designated lab

ELISA: Enzyme-Linked Imunosorbent Assay; **PBMC**: peripheral blood mononuclear cells, ~~**Elispot**: enzyme-linked immunospot assay,~~ **WRAIR**: Walter Reed Army Institute of Research (US), **GVGH**: GSK Vaccines Institute for Global Health.
*GVGH laboratory refers to the GVGH-laboratories in Siena, Italy.

Section 6.7.3, Table 10 Hematology, Serum Chemistry, HLA-B27 test, Urine and Stool tests

EDTA Blood	HLA-B27	~~*PCR*~~***Qualitative flow cytometry***	Study site

PCR: Polymerase Chain Reaction,

Section 11.2 Secondary endpoints (+Synopsis)

Efficacy

VE, in subjects receiving the 1790GAHB vaccine vs. placebo, will be also measured during inpatient stay (Day 57 to Day 64) against:

- Rate of shigellosis ~~(fulfilling~~ ***as defined by*** the CHIM working group case definition for shigellosis~~)~~ occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects.
- […]

- Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [***oral temperature ≥38.0°C OR*** ≥1 severe constitutional/enteric symptom]].
- […]

Section 11.3 Tertiary endpoints (+Synopsis)

- […]
- Frequency of *S. sonnei* LPS specific IgG α4β7+~~/-~~ antibody secreting cells per 10^6 PBMC plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 8, Day 36 and Day 64).
- […]

Section 11.12.1 Sequence of analyses

[…]

~~One interim analysis is intended and will include post-challenge efficacy data of all subjects (all 4 cohorts).~~

The following 2 interim analyses are planned:

1. ***After completion of the Visit 5 (Day 64): post-challenge efficacy data of all subjects (all 4 cohorts)***
2. ***After Visit 6 (Day 85): secondary immunogenicity data and tertiary SBA immunogenicity data of all subjects (all 4 cohorts).***

All analyses will be conducted before unblinding on ***clean and final*** data ~~as clean as possible~~.

An integrated ~~C~~***c***linical ~~S~~***s***tudy ~~R~~***r***eport containing all data will be written ***after the End of Study*** and made available to the investigators.

[…]

Appendix A Laboratory Assays

α4β7 plasmablast ELISA

Anti-S. sonnei LPS-specific IgG titers will be determined in plasmablasts, in both α4β7 positive and α4β7 negative populations. The titers provided will be measured by ELISA assay in the culture supernatant of 5x10^6 cells, by using an in-house method developed in WRAIR.