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

Primary study intervention and number	• altSonflex1-2-3 (GSK4001785A)
Other study interventions	• altSonflex Placebo • Meningococcal (Groups A, C, Y, and W-135) oligosaccharide diphtheria CRM_{197} conjugate vaccine (MENVEO) • Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, adsorbed (BOOSTRIX) • ***Diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis (inactivated), and Haemophilus influenzae type b vaccine (INFANRIX HEXA)*** • Typhoid Vi polysaccharide vaccine (TYPHIM VI) • Measles and rubella vaccine (live) (MR-VAC, Serum Institute of India)
eTrack study number and abbreviated title	212149 (SHIGELLA 4 GMMA GVGH-001) (H06_01TP)
EudraCT number	2021-000891-12
Date of protocol	Final: 26 May 2021
Date of protocol amendment	Amendment 1 Final: 23 September 2021 Amendment 2 Final: 14 February 2022 Amendment 3 Final: 7 February 2023 Amendment 4 Final: 31 Oct 2023
Title	A staged Phase I/II observer-blind, randomised, controlled, multi-country study to evaluate the safety, reactogenicity, and immune responses to the GVGH altSonflex1-2-3 vaccine against *S. sonnei* and *S. flexneri*, serotypes 1b, 2a, and 3a, in adults in Europe (Stage 1) followed by age de-escalation from adults to children and infants, and dose-finding in infants in Africa (Stage 2)
Brief title	A study on the safety and immune responses to the GVGH altSonflex1-2-3 vaccine against shigellosis in adults, children, and infants

Based on GlaxoSmithKline Biologicals SA Protocol WS v17.1

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Protocol Amendment 4 Sponsor Signatory Approval

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Sponsor signatory	Ashwani Kumar Arora, GVGH Head of Clinical Development & Regulatory Affairs
Signature	_____
Date	_____

Note: Not applicable if an alternative signature process (e.g., electronic signature or email approval) is used to get the sponsor approval.

Protocol Amendment 4 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 SA.
- 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.
- That I will comply with the terms of the site agreement.
- To ensure that all persons assisting me with the study are adequately informed about the GSK study intervention and other study-related duties and functions as described in the protocol.
- To ensure that vaccine dilution and possibly necessary labelling of clinical study materials at the study site will be performed according to GSK Biologicals instructions only by trained site staff with appropriate qualifications as required by local laws and regulations.
- To supervise any individual or party to whom I have delegated trial-related duties and functions conducted at the trial site.
- To ensure that any individual or party to whom I have delegated trial-related duties and functions conducted at the trial site are qualified to perform those trial-related duties and functions.
- 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 and the express written informed consent of the participant and/or the participant's legally acceptable representative (LAR).
- To perform no biological assays on the clinical samples other than those described in the protocol or its amendment(s).
- To co-operate with representative(s) of GSK in the monitoring process of the study and in resolution of queries about the data.
- To have control of all essential documents and records generated under my responsibility before, during, and after the trial.
- 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 intervention(s), and more generally about his/her financial ties with the sponsor. GSK will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence, I:

- Agree to supply GSK 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 study and for 1 year following completion of the study.
- Agree that GSK may disclose any information about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK with an updated Curriculum Vitae and all other documents required by regulatory agencies for this study.

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Investigator name	_____
Signature	_____
Date	_____

SPONSOR INFORMATION

1. Sponsor

GlaxoSmithKline Biologicals SA (GSK).

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 Events (SAEs)

GSK central back up study contact for reporting SAEs: refer to the protocol Section 8.3.3.1.

Study contact for reporting SAEs: refer to the local study contact information document.

5. GSK Helpdesk for emergency unblinding

Refer to the protocol section 6.3.6.1.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

Amendment 4 (31 Oct 2023):

This amendment is considered substantial based on the criteria defined in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and the Guidelines for the Conduct of Clinical Trials in Kenya (PPB/MIP/CTL/GUD/003, Chapter 23, 23.3.1.6 and 23.3.1.7) because it impacts the safety of participants.

Overall rationale for the current Amendment:

The amendment has been prepared to improve safety monitoring of participants by modifying the creatinine grading scale using a range that better represents the population being evaluated and by introducing blood urea, sodium, and potassium testing to ensure further monitoring of renal function. Serology sampling has been optimised to obtain more representative results with reference ELISA testing and focus on immune response characterization. Due to supply constraints, the trivalent INFANRIX vaccine is also being substituted with the hexavalent INFANRIX HEXA vaccine providing additional potential benefit to the participants.

List of main changes in the protocol and their rationale:

Section # and title	Description of change	Brief rationale
Cover page; Section 1.3, (Schedule of Activities), Table 4 (Schedule of Activities for infants 9 months of age in Stage 2); Section 2.3 (Benefit/Risk assessment); Section 2.3.3 (Overall Benefit: Risk conclusion); Section 4.1 (Overall design), Table 10 (Study groups, intervention and blinding in infants in Stage 2); Section 4.2.3 (Rationale for choice of control vaccines); Section 6.1 (Study interventions administered), Table 11 (Study interventions administered); Section 8.2.2.1.2 (Stage 2 [Africa])	Replacement of INFANRIX with INFANRIX HEXA as comparator vaccine.	Due to supply shortages of the INFANRIX vaccine, INFANRIX HEXA will be used instead as a comparator to provide additional benefit to participants in the control group.
Section 1.1 (Synopsis); Section 3 (Objectives and endpoints), Table 6 (Study objectives and endpoints);	Clarification that natural exposure to *Shigella* will be tested only in Stage 2 infant participants.	This tertiary objective will be performed only on samples from the target population.
Section 1.1 (Synopsis); Section 3 (Objectives and endpoints), Table 6 (Study objectives and endpoints); Section 8.1.2 (Laboratory assays), Table 15 (Laboratory assays); Section 8.1.3 (Immunological read-outs), Table 16 (Immunological read-outs); Section 9.4.3 (Tertiary endpoints); Section 10.2 (Appendix 2: Clinical laboratory tests)	Replacement of reference ELISA testing of Stage 1 samples with testing of Stage 2 samples from adults, children and infants in safety cohorts up to 28 days after each vaccination; addition of corresponding endpoints.	Samples will be tested in parallel with those tested for secondary immunogenicity objectives and will be more representative of the target population. Additional endpoints will allow comparisons of results between GVGH and reference ELISA in Stage 2

Section # and title	Description of change	Brief rationale
Section 6.1 (Study interventions administered), Table 11 (Study interventions administered); Section 10.10.2 (Glossary of terms); Section 11 (References)	Addition of definitions which are relevant to comparator vaccines to the Glossary of terms; addition of the comparator vaccine product information to the list of references.	This change is in response to a corrective and preventive action aimed to improve the complete and correct identification of comparator product information in GSK protocols.
Section 8.2.2.1.2 (Stage 2 [Africa])	Clarification on the interval of vaccination between the first 5 participants and subsequent participants.	Additional instruction is being provided to the site staff.
Section 8.2.2.3 (Study holding rules), Table 18 (Study holding rules)	Clarification on the planning of blinded monitoring to evaluate the total number of participants vaccinated at the time of the safety evaluation.	This will ensure consistency in the way blinded monitoring outputs are received for review.
Section 9.5.1 (Sequence of analyses)	Clarification that available tertiary endpoint data will be included in interim analyses.	This will allow for the planning of the CSR contents.
Section 10.2.1 (Protocol required safety laboratory assessments), Table 27 (Protocol required safety laboratory assessments)	Addition of blood urea, sodium, and potassium to the pre/post vaccination safety laboratory assays performed in infants.	These additional parameters will be used to ensure adequate monitoring of renal function considering the modification of the creatinine grading scale (below). No additional blood will be collected for these tests.
Section 10.8.4 (Stage 2 – Infants [Africa])	Modification of the creatinine grading scale to better reflect the population being evaluated.	The previous creatinine grading scale did not accurately reflect the potential severity of renal impairment in the child and infant participants in Stage 2 (Kenya), which led to the repeated triggering of holding rules 2b for clinically non-significant events. Low creatinine baseline values were observed in this population, which may be due to borderline asthenia and borderline nutritional deficiency status.
Some formatting and styling errors identified throughout the Protocol were corrected. These have no impact on the content or meaning of the text.		

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1. PROTOCOL SUMMARY

1.1. Synopsis

Rationale: An important objective for GSK is to develop a multicomponent *Shigella* vaccine to prevent the burden of shigellosis in infants and children in developing countries where shigellosis is endemic. Currently, there are no widely used vaccines against *S. sonnei* and *S. flexneri* serotypes despite several attempts in the last decades. GSK Vaccines Institute for Global Health (GVGH) has developed a new candidate *Shigella* 4-component generalised modules for membrane antigens (GMMA) vaccine (GSK4001785A or altSonflex1-2-3) against *S. sonnei* and *S. flexneri* serotypes 1b, 2a, and 3a, which is based on the previously developed GVGH monovalent *S. sonnei* vaccine (1790GAHB vaccine) using the GMMA-platform technology. The current clinical study will evaluate this candidate vaccine, altSonflex1-2-3, for the first time in humans (FTIH).

Objectives and endpoints:

Objectives	Endpoints
Primary	
• To identify the preferred dose of each component of the altSonflex1-2-3 vaccine (low, medium, or high) for infants 9 months of age in Africa (Stage 2).	• Anti-serotype specific *Shigella* lipopolysaccharide (LPS)/O-Antigen (OAg)* serum immunoglobulin G (IgG) geometric mean concentrations (GMC), as measured by GVGH enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the third vaccination (Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the safety and reactogenicity of the altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with unsolicited adverse events (AEs) during 28 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with serious adverse events (SAEs) during their entire study participation period (from Day 1 to Day113/Day 197). • Number of adults 18 to 50 years of age in Europe with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92/Day 176). • Number of adults 18 to 50 years of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85).

Objectives	Endpoints
	• Number of adults 18 to 50 years of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of adults 18 to 50 years of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of children 24 to 59 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of children 24 to 59 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of infants 9 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 281). • Number of infants 9 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8, Day 92, and Day 260).
Secondary	
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Europe (Stage 1).	• Anti-serotype specific *Shigella* LPS/O-Antigen (OAg)* serum IgG geometric mean concentrations (GMCs), in adults 18 to 50 years of age in Europe, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Africa (Stage 2).	• Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in adults 18 to 50 years of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti- serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in children 24 to 59 months of age in Africa, as measured by GVGH

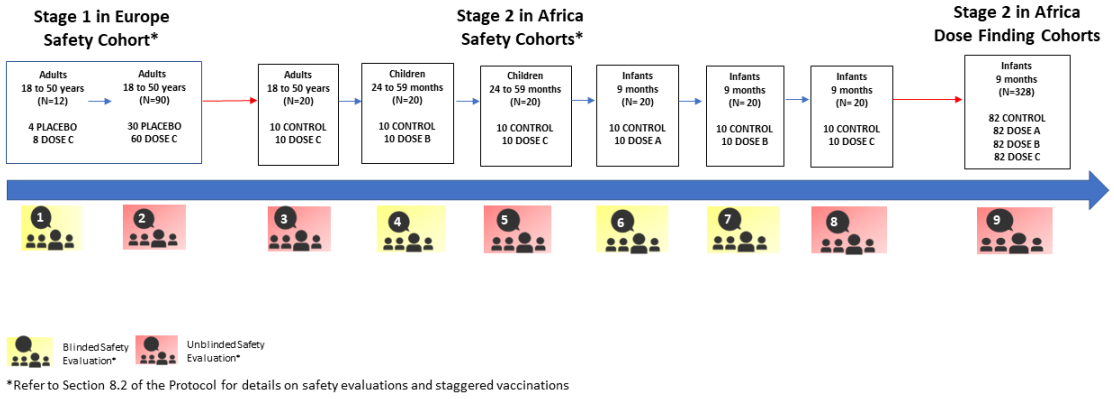
Objectives	Endpoints
	ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in infants 9 months of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
To further characterise the immunogenicity profile of the *S. sonnei* component of altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). *This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the Statistical Analysis Plan (SAP). **This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

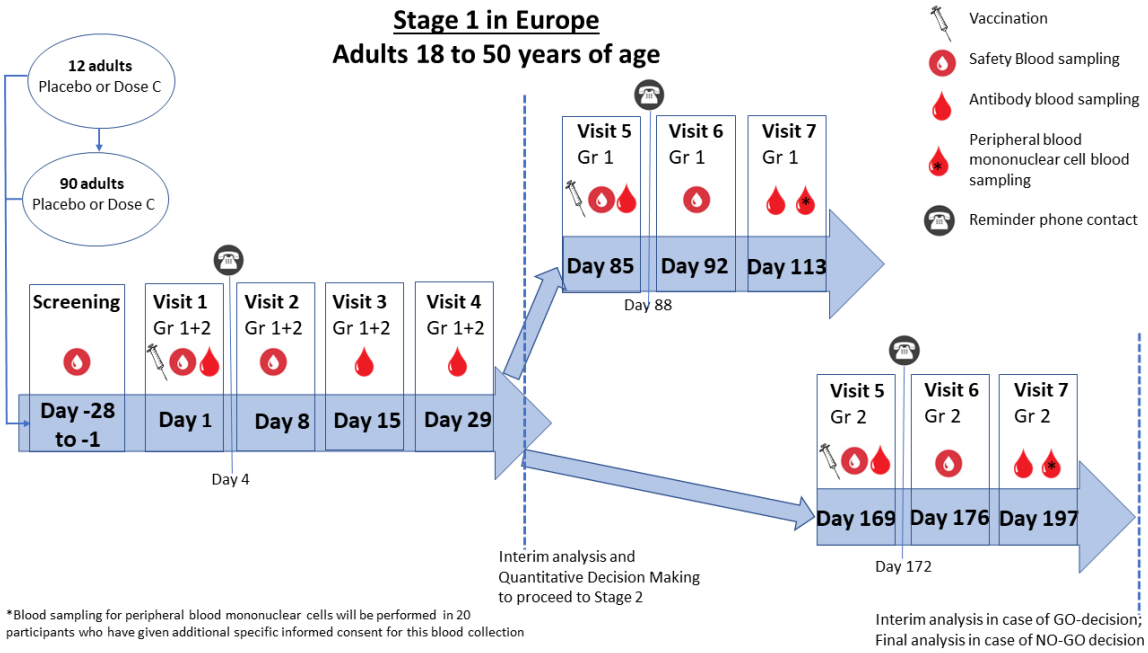
Objectives	Endpoints
• To evaluate seroresponse with the altSonflex1-2-3 vaccine after each vaccination in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 14 days after first vaccination (Day 15) and at 28 days after each vaccination (Day 29 and Day 113/Day 197), compared to baseline (Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Africa showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85). • Number of children 24 to 59 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85). • Number of infants 9 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29, Day 113, and Day 281), compared to baseline (Day 1, Day 85, and Day 253). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity of co-administered MR-VAC in infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Anti-measles IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Anti-rubella IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-measles IgG concentrations of ≥150 mIU/mL* and ≥ 200 mIU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-rubella IgG concentrations of ≥4 IU/mL* and ≥10 IU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). *This threshold is based on the vaccine correlate of protection and World Health Organization guidelines.
Tertiary	
• To evaluate the immune response induced by the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1), subsets of adults, children, and infants in the safety cohorts, and all infants in the dose-finding cohort, in Africa (Stage 2), using the serum bactericidal assay (SBA).	• Anti-serotype specific* serum bactericidal antibody geometric meant titres (GMTs) in 25% of adults in Europe 18 to 50 years of age, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of adults in Africa 18 to 50 years of age, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113).

Objectives	Endpoints
	• Anti-serotype specific* serum bactericidal antibody GMTs in a subset of children 24 to 59 months of age before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of infants 9 months of age in the safety cohort, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Anti-serotype specific* serum bactericidal antibody GMTs in ***all*** infants 9 months of age in the dose-finding cohort, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and a subset of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Antibody features (subclassing, affinity, avidity) and functional properties (induction of cellular phagocytosis and cellular cytotoxicity, natural killer [NK] cell activation, eosinophil and basophil degranulation, complement deposition, neutrophil activation/phagocytosis, dendritic cell phagocytosis, mucin binding, glycosylation, and Fc receptor alteration), as measured by systems serology analysis. • Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous *Shigella* serotypes, as measured by SBA. • Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine homologous and heterologous *Shigella* serotypes, as measured by opsonophagocytosis assay.
• To measure natural exposure to *Shigella* in Stage 2 ***infant*** participants	• Anti-*Shigella*-conserved protein IgG, as measured by anti-protein ELISA.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ***adult, child, and infant safety cohorts,*** in ***Africa (Stage 2)***.	• Anti-serotype specific *Shigella* LPS* serum IgG GMTs, in adults 18 to 50 years of age in ***Africa***, as measured by reference ELISA, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in children 24 to 59 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in infants 9 months of age in the safety cohorts in Africa, as measured by reference ELISA, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:800** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113).*** • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).***

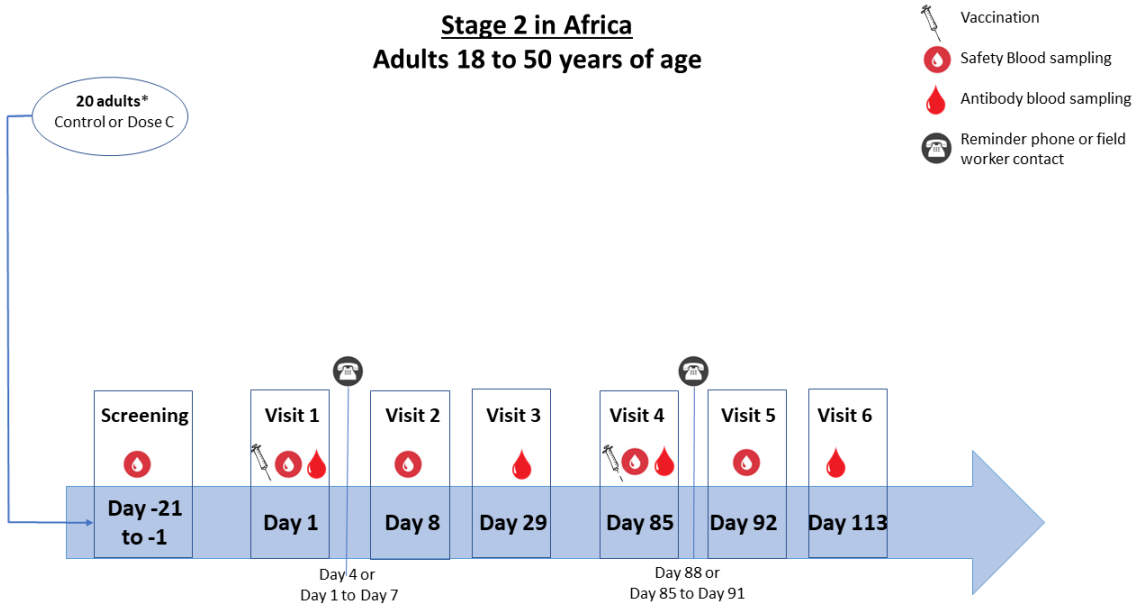
Objectives	Endpoints
	• ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:1600*** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested. **This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. ***This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

1.2. Schema

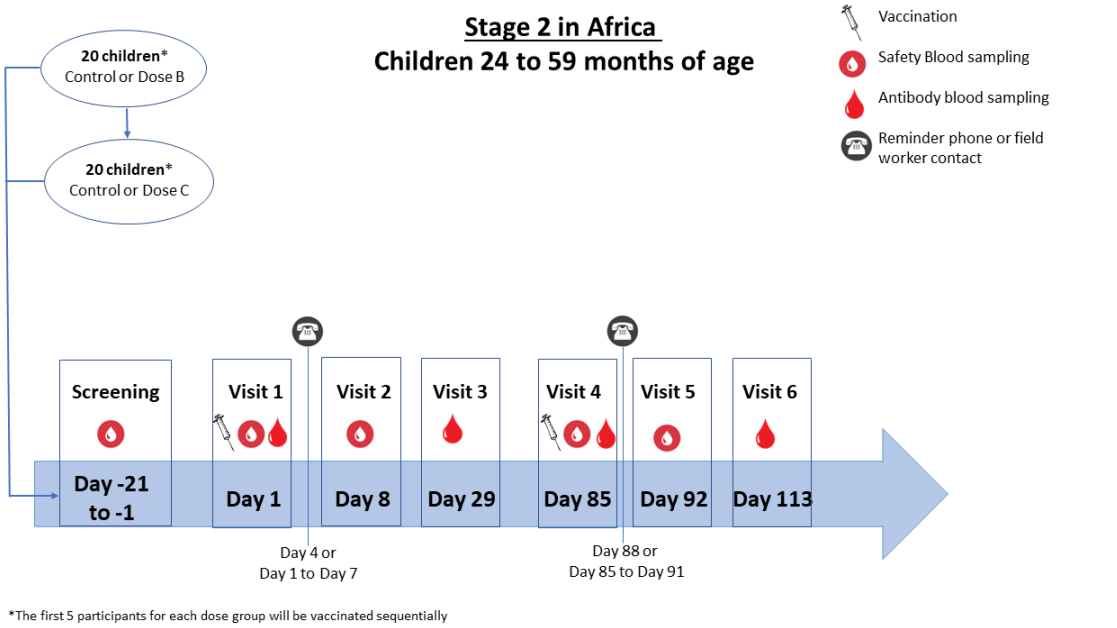




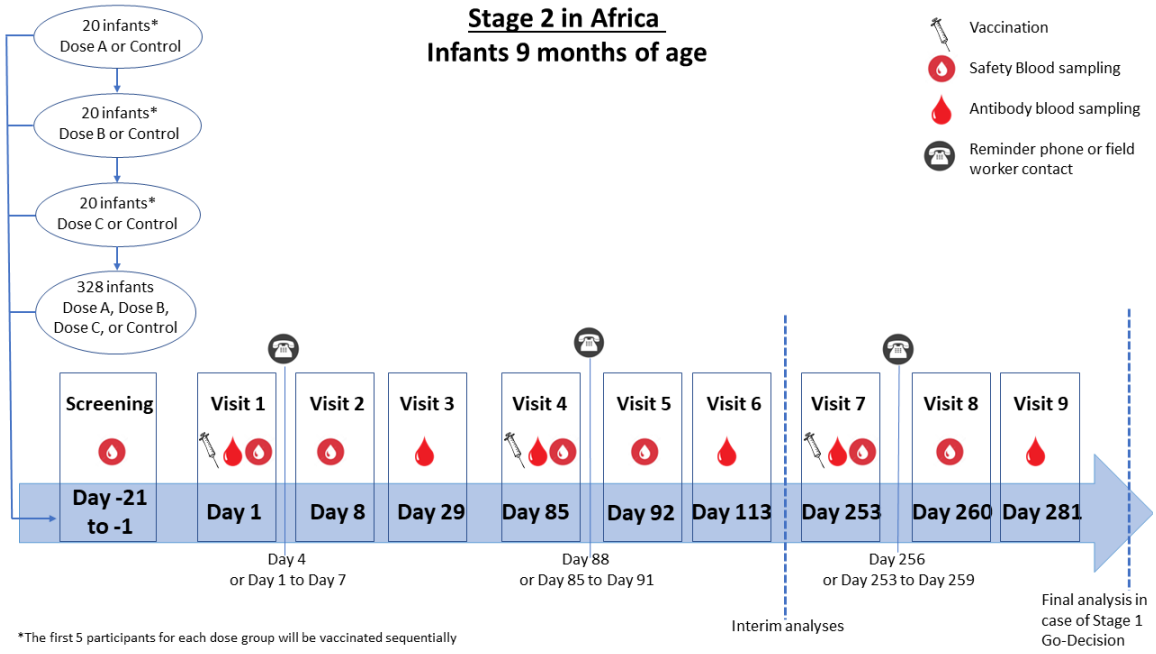
*Blood sampling for peripheral blood mononuclear cells will be performed in 20 participants who have given additional specific informed consent for this blood collection



*The first 5 participants will be vaccinated sequentially



*The first 5 participants for each dose group will be vaccinated sequentially



*The first 5 participants for each dose group will be vaccinated sequentially

1.3. Schedule of Activities (SoA)

Table 1 Schedule of Activities for Stage 1

Age		18 to 50 years												
Type of contact	Screening period	Visit 1	Reminder phone contact	Visit 2	Visit 3	Visit 4	Visit 5		Reminder phone contact		Visit 6		Visit 7	
Timepoints	Day -28 to Day -1	Day 1	Day 4	Day 8	Day 15	Day 29	Day 85	Day 169	Day 88	Day 172	Day 92	Day 176	Day 113	Day 197
Group(s)	Group 1 and 2	Group 1 and 2	Group 1 and 2	Group 1 and 2	Group 1 and 2	Group 1 and 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Informed consent (Section 10.1.3)	●													
Check inclusion/exclusion criteria (Sections 5.1 and 5.2)	●	●					●							
Collect demographic data (Section 8.2.1.1)	●													
Medical history (Section 8.2.1.2)	●													
Physical examination (including vital sign assessment and body temperature measurement) (Section 8.2.1.3)	●	●					●							
Symptom-directed physical examination (Section 8.2.1.4)				○	○	○					○		○	
Height and weight measurements (Section 8.2.1.3)	●													
Study intervention														
Check contraindications and warnings, and precautions to vaccination (Sections 7.1.1 and 8.2.1.5)	○	○					○							
Check criteria for temporary delay for enrolment and/or study intervention administration (Section 5.5)	○	○					○							
Urine pregnancy test (Section 8.2.1.4)	●	●					●							
Study group and study intervention number allocation (Section 6.3)		○												
Study intervention number retrieval for subsequent doses (Section 6.3)							○							

Age		18 to 50 years												
Type of contact	**Screening period**	**Visit 1**	**Reminder phone contact**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**		**Reminder phone contact**		**Visit 6**		**Visit 7**	
Timepoints	**Day -28 to Day -1**	**Day 1**	**Day 4**	**Day 8**	**Day 15**	**Day 29**	**Day 85**	**Day 169**	**Day 88**	**Day 172**	**Day 92**	**Day 176**	**Day 113**	**Day 197**
Group(s)	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**
Recording of administered study intervention number (Section 6.3)		●					●							
AltSonflex1-2-3 or altSonflex Placebo administration (Section 6.1)		●					●							
Laboratory assessments														
Blood sampling for antibody determination (~10 mL) (Section 8.1.1)		●			●	●	●						●	
Blood sampling for assay development (~10 mL) (Section 8.1.1)													●	
Blood sampling for safety screening laboratory assays (haematology panel, clinical chemistry, viral serologic analyses, and HLA-B27 testing) (~15 mL) (Section 8.1.1)	●													
Blood sampling for safety laboratory assays (haematology panel and clinical chemistry analyses) (~10 mL) (Section 8.1.1)		●		●			●				●			
Blood sampling for PBMC isolation and serum collection (~up to 150 mL) (Section 8.1.1)													●[1,2]	
Safety assessments														
Record any concomitant medication/vaccination (Section 6.8)	●	●		●	●	●	●				●		●	
Record any intercurrent medical conditions (Section 9.3.1)		●		●	●	●	●				●		●	
Recording of solicited events in paper Diary (Days 1-7 post-vaccination) (Section 10.3.8)		○					○							

Age		18 to 50 years												
Type of contact	**Screening period**	**Visit 1**	**Reminder phone contact**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**		**Reminder phone contact**		**Visit 6**		**Visit 7**	
Timepoints	**Day -28 to Day -1**	**Day 1**	**Day 4**	**Day 8**	**Day 15**	**Day 29**	**Day 85**	**Day 169**	**Day 88**	**Day 172**	**Day 92**	**Day 176**	**Day 113**	**Day 197**
Group(s)	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1 and 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**	**Group 1**	**Group 2**
Recording of unsolicited AEs in the source document (Days 1-28 post-vaccination) (Section 10.3.8)		○		○	○		○				○			
Transcription of unsolicited AEs in the eCRF by the investigator or delegate				●		●					●		●	
Safety surveillance (including vital sign assessment and body temperature measurement) (60 minutes post-vaccination) (Section 10.3.8)		●					●							
Reporting of SAEs, pregnancies, and pregnancy outcomes (Section 10.3.8)	●	●		●	●	●	●				●		●	
Diary cards														
Distribution of diary cards		○					○							
Phone call for diary card completion reminder (Section 10.3.8)			●						●					
Diary card transcription in the eCRF by investigator or delegate				●							●			
Return of diary cards				●							●			
Participant study conclusion (Section 4.4)													●	

AE=adverse event; eCRF=electronic Case Report Form; HLA-B27=human leukocyte antigen B27; PBMC=peripheral blood mononuclear cell; SAE=Serious Adverse Event
Note: The double-line borders following Day 29 indicate interim analyses in these participants, which will be performed on all data obtained up to Day 29. The double-line borders following Day 197 indicate interim or final analyses in these participants, which will be performed on all data obtained up to Day 197.
● is used to indicate a study procedure that requires documentation in the individual electronic Case Report Form (eCRF).
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1]Extracted PBMC and sera prepared from collected blood will be used in exploratory research for the isolation, identification, and characterisation of human monoclonal antibodies against *Shigella.*
[2]Blood will be collected in the first 20 participants who reach Visit 7 and have given specific consent for this collection.

Table 2 Schedule of Activities for Adults 18 to 50 years of age in Stage 2

Age		18 to 50 years									
Type of contact	**Screening period**	**Visit 1**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 2**	**Visit 3**	**Visit 4**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 5**	**Visit 6**
Time points	**Day -21 to Day -1**	**Day 1**	**Day 4**	**Day 1 to Day 7**	**Day 8**	**Day 29**	**Day 85**	**Day 88**	**Day 85 to Day 91**	**Day 92**	**Day 113**
Informed consent (Section 10.1.3)	●										
Check inclusion/exclusion criteria (Sections 5.1 and 5.2)	●	●					●				
Collect demographic data (Section 8.2.1.1)	●										
Medical history (Section 8.2.1.2)	●										
Physical examination (including vital sign assessment and body temperature measurement) (Section 8.2.1.3)	●	●					●				
Symptom-directed physical examination (Section 8.2.1.3)					○	○				○	○
Height and weight measurements (Section 8.2.1.3)	●										
Study interventions											
Check contraindications and warnings and precautions to vaccination (Sections 7.1.1 and 8.2.1.5)	○	○					○				
Check criteria for temporary delay for enrolment and/or study intervention administration (Section 5.5)	○	○					○				
Urine pregnancy test (Section 8.2.1.4)	●	●					●				
Body temperature measurement					○	○				○	○
Study group and study intervention number allocation (Section 6.3)		○									
Study intervention number retrieval for subsequent doses (Section 6.3)							○				
Recording of administered study intervention number (Section 6.3)		●					●				

Age		18 to 50 years									
Type of contact	**Screening period**	**Visit 1**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 2**	**Visit 3**	**Visit 4**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 5**	**Visit 6**
Time points	**Day -21 to Day -1**	**Day 1**	**Day 4**	**Day 1 to Day 7**	**Day 8**	**Day 29**	**Day 85**	**Day 88**	**Day 85 to Day 91**	**Day 92**	**Day 113**
AltSonflex1-2-3 or Control vaccine administration (Section 6.1)		●[3]					●[4]				
Laboratory assessments											
Blood sampling for antibody determination (~10 mL) (Section 8.1.1)		●				●	●				●
Blood sampling for safety screening laboratory assays (haematology panel, clinical chemistry, and viral serologic analyses, and HLA-B27) (~12 mL) (Section 8.1.1)	●										
Blood sampling for safety laboratory assays (haematology panel and clinical chemistry analyses) (~6 mL) (Section 8.1.1)		●			●		●			●	
Safety assessments											
Record any concomitant medication/vaccination (Section 6.8)	●	●			●	●	●			●	●
Record any intercurrent medical conditions (Section 9.3.1)		●			●	●	●			●	●
Recording of solicited events in paper Diary (Days 1-7 post-vaccination) (Section 10.3.8)		○		○[5]			○		○[5]		
Recording of unsolicited AEs in the source document (Days 1-28 post-vaccination) (Section 10.3.8)		○			○		○			○	
Transcription of unsolicited AEs in the eCRF by the investigator or delegate					●	●				●	●
Safety surveillance (including vital sign assessment and body temperature measurement) (60 minutes post-vaccination) (Section 10.3.8)		●					●				

Age		18 to 50 years									
Type of contact	**Screening period**	**Visit 1**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 2**	**Visit 3**	**Visit 4**	**Reminder phone contact[1]**	**Field worker daily home visit[2]**	**Visit 5**	**Visit 6**
Time points	**Day -21 to Day -1**	**Day 1**	**Day 4**	**Day 1 to Day 7**	**Day 8**	**Day 29**	**Day 85**	**Day 88**	**Day 85 to Day 91**	**Day 92**	**Day 113**
Reporting of SAEs, pregnancies, and pregnancy outcomes (Section 10.3.8)	●	●			●	●	●			●	●
Diary cards											
Distribution of diary cards		○					○				
Phone call for diary card completion reminder to literate participants (Section 10.3.8)			●					●			
Field worker visit for diary card completion for illiterate participants (Section 10.3.8)				●					●		
Diary card transcription in eCRF by investigator or delegate					●					●	
Return of diary cards					●[6]					●[6]	
Participant study conclusion (Section 4.4)											●

AE=adverse event; eCRF=electronic Case Report Form; HLA-B27=human leukocyte antigen B27; SAE=Serious Adverse Event
● is used to indicate a study procedure that requires documentation in the individual electronic Case Report Form (eCRF).
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1]For literate participants only.
[2]For illiterate participants only.
[3]The first control vaccination will consist of the MENVEO vaccine.
[4]The second control vaccination will consist of the BOOSTRIX vaccine.
[5] For illiterate participants, diary cards will be completed by the site field worker.
[6] For illiterate participants, diary cards can be returned by the field worker at the end of the 7 days after vaccination.

Table 3 Schedule of Activities for Children 24 to 59 months of age in Stage 2

Age		24-59 months				25-60 months	27-62 months				28-63 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113
Informed consent by parent(s)/legally acceptable representative(s) (LAR(s)) (Section 10.1.3)	●										
Check inclusion/exclusion criteria (Sections 5.1 and 5.2)	●	●					●				
Collect demographic data (Section 8.2.1.1)	●										
Medical and vaccination history (Section 8.2.1.2)	●										
Physical examination (including vital sign assessment and body temperature measurement) (Section 8.2.1.3)	●	●					●				
Symptom-directed physical examination (Section 8.2.1.4)					○	○				○	○
Height and weight measurements (Section 8.2.1.3)	●	○			○	○	○			○	○
Study interventions											
Check contraindications and warnings, and precautions to vaccination (Sections 7.1.1 and 8.2.1.5)	○	○					○				
Check criteria for temporary delay for enrolment and/or study intervention administration (Section 5.5)	○	○					○				
Body temperature measurement					○	○				○	○
Study group and study intervention number allocation (Section 6.3)		○									
Study intervention number retrieval for subsequent doses (Section 6.3)							○				

Age		24-59 months				25-60 months	27-62 months				28-63 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113
Recording of administered study intervention number (Section 6.3)		●					●				
AltSonflex1-2-3 Vaccine or Control vaccine administration (Section 6.1)		●[3]					●[4]				
Laboratory assessments											
Blood sampling for antibody determination (~10 mL) (Section 8.1.1)		●				●	●				●
Blood sampling for safety screening laboratory assays (haematology panel, clinical chemistry, viral serologic analyses, and HLA-B27) (~10 mL) (Section 8.1.1)	●										
Blood sampling for safety laboratory assays (haematology panel and clinical chemistry analyses) (~6 mL) (Section 8.1.1)		●			●		●			●	
Safety assessments											
Record any concomitant medication/vaccination (Section 6.8)	●	●			●	●	●			●	●
Record any intercurrent medical conditions (Section 9.3.1)		●			●	●	●			●	●
Recording of solicited events in paper Diary (Days 1-7 post-vaccination) (Section 10.3.8)		○		○[5]			○		○[5]		
Recording of unsolicited AEs in the source document (Days 1-28 post-vaccination) (Section 10.3.8)		○			○		○			○	
Transcription of unsolicited AEs in the eCRF by the investigator or delegate					●	●				●	●

Age		24-59 months				25-60 months	27-62 months				28-63 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113
Safety surveillance (including vital sign assessment and body temperature measurement) (60 minutes post-vaccination) (Section 10.3.8)		●					●				
Reporting of SAEs (Section 10.3.8)	●	●			●	●	●			●	●
Diary cards											
Distribution of diary cards		○					○				`
Phone call for diary card completion reminder to literate parent(s)/LAR(s) (Section 10.3.8)			●					●			
Field worker visit for diary card completion for illiterate parent(s)/LAR(s) (Section 10.3.8)				●					●		
Diary card transcription in the eCRF by investigator or delegate					●					●	
Return of diary cards					●[6]					●[6]	
Participant study conclusion (Section 4.4)											●

AE=adverse event; eCRF=electronic Case Report Form; HLA-B27=human leukocyte antigen B27; LAR=Legally Acceptable Representative; SAE=Serious Adverse Event

● is used to indicate a study procedure that requires documentation in the individual electronic Case Report Form (eCRF).

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

[1]For literate parent(s)/LAR(s) only

[2]For illiterate parent(s)/LAR(s) only

[3]The first control vaccination will consist of the MENVEO vaccine.

[4]The second control vaccination will consist of the TYPHIM VI vaccine.

[5]For illiterate parent(s)/LAR(s), diary cards will be completed by the site field worker.

[6]For illiterate parent(s)/LAR(s), diary cards can be returned by the field worker at the end of the 7 days after vaccination.

Table 4 Schedule of Activities for Infants 9 months of age in Stage 2

Age		9 months				10 months	12 months				13 months	18 months				19 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6	Visit 7	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 8	Visit 9
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113	Day 253	Day 256	Day 253 to Day 259	Day 260	Day 281
Informed consent by parent(s)/legally acceptable representative (LAR(s)) (Section 10.1.3)	●															
Check inclusion/exclusion criteria (Sections 5.1 and 5.2)	●	●					●					●				
Collect demographic data (Section 8.2.1.1)	●															
Medical and vaccination history (Section 8.2.1.2)	●															
Physical examination (including vital sign assessment and body temperature measurement) (Section 8.2.1.3)	●	●					●					●				
Symptom-directed physical examination (Section 8.2.1.3)					○	○				○	○				○	○
Weight and length measurements (Section 8.2.1.3)	●	○			○	○	○			○	○	○			○	○

Age		9 months				10 months	12 months				13 months	18 months				19 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6	Visit 7	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 8	Visit 9
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113	Day 253	Day 256	Day 253 to Day 259	Day 260	Day 281
Study interventions																
Check contraindications and warnings, and precautions to vaccination (Sections 7.1.1 and 8.2.1.5)	○	○				○	○					○				
Check criteria for temporary delay for enrolment and/or study intervention administration (Section 5.5)	○	○					○					○				
Body temperature measurement					○	○				○	○				○	○
Study group and study intervention number allocation (Section 6.3)		○														
Study intervention number retrieval for subsequent doses (Section 6.3)							○					○				
Recording of administered study intervention number (Section 6.3)		●					●					●				
AltSonflex1-2-3 or Control vaccine administration (Section 6.1)		●[3]					●[3]					●[4]				
MR-VAC administration in the safety cohort participants (Section 6.1)						●										●

Age		9 months				10 months	12 months				13 months	18 months				19 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6	Visit 7	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 8	Visit 9
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113	Day 253	Day 256	Day 253 to Day 259	Day 260	Day 281
MR-VAC administration in the dose-finding cohort (Section 6.1)		●										●				
Laboratory assessments																
Blood sampling for antibody determination (~5 mL) (Section 8.1.1)		●				●	●				●	●				●
Blood sampling for safety screening laboratory assays (haematology panel, clinical chemistry, viral serologic analyses, and HLA-B27) (~9 mL) (Section 8.1.1)	●															
Blood sampling for safety laboratory assays (haematology panel and clinical chemistry analyses) (~2 mL) (Section 8.1.1)		●			●		●			●		●			●	
Safety assessments																
Record any concomitant medication/vaccination (Section 6.8)	●	●			●	●	●			●	●	●			●	●
Record any intercurrent medical conditions (Section 9.3.1)		●			●	●	●			●	●	●			●	●

Age		9 months				10 months	12 months				13 months	18 months				19 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6	Visit 7	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 8	Visit 9
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113	Day 253	Day 256	Day 253 to Day 259	Day 260	Day 281
Recording of solicited events in paper Diary (Days 1-7 post-vaccination) (Section 10.3.8)		○		○[5]			○		○[5]			○		○[5]		
Recording of unsolicited AEs in the source document (Days 1-28 post-vaccination) (Section 10.3.8)		○			○		○			○		○			○	
Transcription of unsolicited AEs in the eCRF by the investigator or delegate					●	●				●	●				●	●
Safety surveillance (including vital sign assessment and body temperature measurement) (60 minutes post-vaccination) (Section 10.3.8)		●					●					●				
Reporting of SAEs (Section 10.3.8)	●	●			●	●	●			●	●	●			●	●

Age		9 months				10 months	12 months				13 months	18 months				19 months
Type of contact	Screening period	Visit 1	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 2	Visit 3	Visit 4	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 5	Visit 6	Visit 7	Reminder phone contact[1]	Field worker daily home visit[2]	Visit 8	Visit 9
Time points	Day -21 to Day -1	Day 1	Day 4	Day 1 to Day 7	Day 8	Day 29	Day 85	Day 88	Day 85 to Day 91	Day 92	Day 113	Day 253	Day 256	Day 253 to Day 259	Day 260	Day 281
Diary cards																
Distribution of diary cards		○					○					○				
Phone call for diary card completion reminder to literate parent(s)/LAR(s) (Section 10.3.8)			●					●					●			
Field worker visit for diary card completion for illiterate parent(s)/LAR(s) (Section 10.3.8)				●					●					●		
Diary card transcription in the eCRF by investigator or delegate					●					●					●	
Return of diary cards					●[6]					●[6]					●[6]	
Participant study conclusion (Section 4.4)																●

AE=adverse event; eCRF=electronic Case Report Form; HLA-B27=human leukocyte antigen B27; LAR=Legally Acceptable Representative; SAE=Serious Adverse Event

Note: The double-line borders following Day 113 indicate interim analyses in the infant safety cohort (interim analysis 3) and in the infant dose-finding cohort (interim analysis 4), which will be separately performed on all data obtained up to Day 113. The double-line borders following Day 281 indicate final analysis of the study, which will be performed on all study data obtained up to Day 281.

● is used to indicate a study procedure that requires documentation in the individual electronic Case Report Form (eCRF).

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

[1]For literate parent(s)/LAR(s) only

[2]For illiterate parent(s)/LAR(s) only

[3]The control vaccination will consist of MENVEO.

[4]The control vaccination will consist of the ***INFANRIX HEXA*** vaccine.

[5]For illiterate parent(s)/LAR(s), diary cards will be completed by the site field worker.

[6]For illiterate parent(s)/LAR(s), diary cards can be returned by the field worker at the end of the 7 days after vaccination.

Table 5 Intervals between study visits

Interval	Planned visit interval	Allowed interval range*
Stage 1 - Adults 18 to 50 years of age - Groups 1 and 2		
Visit 1→Reminder phone contact	3 days	2 days-4 days
Visit 1→Visit 2	7 days	7 days-10 days
Visit 1→Visit 3	14 days	12 days- 21 days
Visit 1→Visit 4	28 days	28 days-33 days
Stage 1 - Adults 18 to 50 years of age - Group 1		
Visit 4→Visit 5	56 days	53 days-59 days
Visit 5→Visit 6	7 days	7 days- 10 days
Visit 5→Reminder phone contact	3 days	2 days-4 days
Visit 5→ Visit 7	28 days	28 days-33 days
Stage 1 - Adults 18 to 50 years of age - Group 2		
Visit 4→Visit 5	140 days	133 days-147 days
Visit 5→Visit 6	7 days	7 days-10 days
Visit 5→Reminder phone contact	3 days	2 days-4 days
Visit 5→ Visit 7	28 days	28 days-33 days
Stage 2 - Adults 18 to 50 years of age		
Visit 1→Reminder phone contact	3 days	2 days-4 days
Visit 1→Visit 2	7 days	7 days-10 days
Visit 1→Visit 3	28 days	28 days-33 days
Visit 3→Visit 4	56 days	53 days-59 days
Visit 4→Visit 5	7 days	7 days-10 days
Visit 4→Reminder phone contact	3 days	2 days-4 days
Visit 4→Visit 6	28 days	28 days-33 days
Stage 2 - Children 24 to 59 months of age		
Visit 1→Reminder phone contact	3 days	2 days-4 days
Visit 1→Visit 2	7 days	7 days-10 days
Visit 1→Visit 3	28 days	28 days-33 days
Visit 3→Visit 4	56 days	53 days-59 days
Visit 4→Reminder phone contact	3 days	2 days-4 days
Visit 4→Visit 5	7 days	7 days-10 days
Visit 4→Visit 6	28 days	28 days-33 days
Stage 2 - Infants 9 months of age		
Visit 1→Reminder phone contact	3 days	2 days-4 days
Visit 1→Visit 2	7 days	7 days-10 days
Visit 1→Visit 3	28 days	28 days-42 days for safety cohort 28 days-33 days for dose-finding cohort
Visit 3→Visit 4	56 days	53 days-66 days
Visit 4→Reminder phone contact	3 days	2 days-4 days
Visit 4→Visit 5	7 days	7 days-10 days
Visit 4→Visit 6	28 days	28 days-42 days for safety cohort 28 days-33 days for dose-finding cohort
Visit 6→Visit 7	140 days	133 days-147 days
Visit 7→Reminder phone contact	3 days	2 days-4 days
Visit 7→Visit 8	7 days	7 days-10 days
Visit 7→Visit 9	28 days	28 days-33 days

*In case a participant is out of window for a particular Visit, the next Visit should be scheduled based on the originally planned protocol visit intervals and allowed interval ranges

2. INTRODUCTION

2.1. Study rationale

An important objective for GSK is to develop a multicomponent *Shigella* vaccine to prevent the burden of shigellosis in infants and children in developing countries where shigellosis is endemic. Currently, there are no widely used vaccines against *S. sonnei* and *S. flexneri* serotypes despite several attempts in the last decades. GSK Vaccines Institute for Global Health (GVGH) has developed a new candidate *Shigella* 4-component generalised modules for membrane antigens (GMMA) vaccine (GSK4001785A or altSonflex1-2-3) against *S. sonnei* and *S. flexneri* serotypes 1b, 2a, and 3a, which is based on the previously developed GVGH monovalent *S. sonnei* vaccine (1790GAHB vaccine) using the GMMA-platform technology. The current clinical study will evaluate this candidate vaccine, altSonflex1-2-3, for the first time in humans (FTIH).

2.2. Background

Shigellosis remains a major public health problem around the world; it is one of the leading causes of diarrhoeal disease in low- and middle-income countries (LMIC), particularly in young children. The Global Burden of Disease study (IHME) estimated that in 2017 *Shigella* caused approximately 238 000 deaths globally and, with 15.2%, it ranked second with regard to pathogen contributions in global diarrhoeal deaths (available from: https://vizhub.healthdata.org/gbd-compare/). In infants, in LMIC, particularly when healthcare resources are limited, shigellosis can often lead to death. In 2016, *Shigella* was responsible for 63 713 deaths among children less than 5 years of age and 212 438 deaths among all ages [GBD, 2018].

There is presently no widely available vaccine against *Shigella*. Treatment for shigellosis is mainly supportive to keep patients stable and restore lost electrolytes due to diarrhoea. If available, specific antibiotics are sometimes used for severe cases of shigellosis. Current drugs are under threat from the increasing rates of antibiotic resistance in *Shigella* serotypes around the world.

The immune response against the O-Antigen (OAg) of bacterial lipopolysaccharide (LPS) of *Shigella* bacteria is known to confer serotype-specific immunity and protection against infection in participants that have recovered from primary infections, as well as those that have been challenged with an infectious dose of the bacteria in human challenge vaccine studies [Cohen, 1991; Ferreccio, 1991]. GVGH has developed and characterised a monovalent vaccine against *S. sonnei* (1790GAHB vaccine) using the GMMA technology. The 1790GAHB vaccine is composed of purified GMMA adsorbed on Alhydrogel. The GMMA are designed to present the OAg portion of *S. sonnei* LPS molecule as an active moiety.

The 1790GAHB vaccine was tested in 2 Phase 1 dose-escalation studies (NCT02017899 and NCT02034500) in Europe (3 vaccinations, 1 month apart via 3 routes of administration: intramuscular [IM], intranasal, and intradermal) and in 1 Phase 1 booster study in France (NCT3089879) for the evaluation of the immunological memory response after a 1-dose booster vaccination with the 1790GAHB vaccine. Results have shown that by the IM route up to 6/100 µg GMMA OAg/proteins has an acceptable

safety profile and induces antibody responses of at least the same magnitude previously observed following natural exposure to the pathogen [Launay, 2017; Cohen, 1988]. A booster injection elicited a strong immune response, regardless of the priming dose [Launay, 2019].

A Phase 2a study (NCT02676895) evaluated the safety and immunogenicity of 2 doses (1.5/25 µg & 6/100 µg OAg/protein) of the *S. sonnei* 1790GAHB vaccine, delivered by IM route to healthy adults from a shigellosis-endemic country, Kenya, with a schedule of 2 vaccinations, 1 month apart. This study confirmed the acceptable safety profile and the immunogenicity induced by the vaccine 28 days after first and second vaccination despite a high baseline antibody concentration [Obiero, 2017]. A Phase 2b *S. sonnei* Controlled Human Infection Model (CHIM) study (NCT03527173) was conducted in the United States at the Cincinnati Children's Hospital Medical Center to evaluate the efficacy, immunogenicity, and safety of the candidate *S. sonnei* 1790GAHB vaccine at a dose of 1.5/25 µg OAg/GMMA proteins. The vaccine confirmed the safety and immunogenicity profiles shown in previous studies but did not show efficacy against *Shigella* infection for the primary objective or any of the clinical endpoints for moderate to severe shigellosis. Interpretation of the results of the CHIM trial identified suboptimal immunogenicity as the most likely root cause for the lack of clinical efficacy and have informed the study design of the current study.

The monovalent *S. sonnei* 1790GAHB vaccine was the stepping-stone for the development of a multicomponent broadly protective *Shigella* 4-component GMMA vaccine (altSonflex1-2-3) for vaccination of infants 9 month of age in LMIC where shigellosis is endemic. Both the *S. sonnei* 1790GAHB vaccine and the altSonflex1-2-3 vaccines consist of purified GMMA adsorbed on Alhydrogel, which present the OAg portion of LPS molecules, and act as the vaccine's active moiety for the principal immune response. The amount of OAg in the altSonflex1-2-3 formulation has been guided by the results of the CHIM study (NCT03527173) and by the dose-response relationship observed in the Phase 1 dose-escalation study in France [Micoli, 2021]. Additionally, the strong booster immune response seen in the extension study has suggested the need to evaluate longer intervals between primary vaccinations with altSonflex1-2-3 in the current Phase 1 Stage of the study. For the *S. sonnei* component, 1790-GMMA used in previous GVGH 1790GAHB trials, will be replaced by the newly developed 2929-GMMA. This alternate construct expresses an increased OAg density and, having a higher OAg to protein and Lipid A ratio than 1790-GMMA, it allows to safely increase the amount of OAg without affecting the safety profile of the vaccine. The altSonflex1-2-3 GMMA vaccine will be tested at a dose containing 15 µg OAg of each serotype.

The current FTIH clinical study will evaluate the safety and immunogenicity of the altSonflex1-2-3 GMMA candidate vaccine against *S. sonnei* and *S. flexneri* serotypes 1b, 2a, and 3a. The candidate altSonflex1-2-3 vaccine will be administered intramuscularly first in adults 18 to 50 years of age in Europe and, subsequently, to adults 18 to 50 years of age, children 24 to 59 months of age, and infants 9 months of age from a shigellosis-endemic population in Africa, using 3 different doses of the vaccine (A [low], B [medium], and C [high]). The highest vaccine dose (Dose C) will be a dose that has been safely tested in toxicological studies and will contain 15 µg of each *S. sonnei* and *S.*

flexneri 1b, 2a, 3a GMMA. The results of this study will allow the selection of the most appropriate dose for further vaccine development in infants 9 months of age, which is the main target age group for this vaccine. Since the main burden of shigellosis is in children below 5 years of age in developing countries (with a peak in the second and third year of life), it is expected that a broadly protective *Shigella* vaccine will be used in young children in shigellosis-endemic countries at the age of 9 months, concomitantly with other Expanded Program on Immunisation (EPI) vaccines, such as measles mumps, rubella or yellow fever depending on a country's EPI schedule.

Please refer to the current Investigator's Brochure (IB) for information regarding preclinical studies of altSonflex1-2-3.

2.3. Benefit/Risk assessment

Detailed information about the known and expected benefits and risks, and expected adverse events (AEs) of altSonflex1-2-3 can be found in the Investigator Brochure.

Detailed information about the known and expected benefits and risks, and expected AEs of MENVEO, BOOSTRIX, TYPHIM VI, MR-VAC**,** and ***INFANRIX HEXA*** can be found in the Prescribing Information/Summary of Product Characteristics.

2.3.1. Risk assessment

Important/Potential/ Identified/Risk of Clinical Significance	Summary of Data/ Rationale for Risk	Mitigation Strategy
altSonflex1-2-3 (GSK4001785A)		
Important potential risk: Anaphylaxis	Anaphylaxis is considered a potential risk since vaccines are a mixture of compounds and allergic sensitisation can occur to any component. Participants may be sensitised to the vaccine antigens, adjuvants, excipients used in the manufacturing process, or a latex stopper on the vial [Erlewyn-Lajeunesse, 2007]. However, the incidence of anaphylaxis is generally very low, and estimates are in the range of 1–10 per 1 million doses distributed depending on the vaccine studied [Rüggeberg, 2007].	Participants will be closely monitored for any symptoms of anaphylaxis. Medical treatment will be readily available in case of anaphylactic reactions following vaccine/control/placebo administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis will be available.
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 minimise 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 (ICF). The amount of blood to be taken for sampling is within the locally accepted range for the age groups and is not expected to be harmful to the participant's health.

General risks of clinical significance related to the study vaccines and precautions:

As with any vaccines:

- Vaccination should be postponed in participants suffering from an acute severe febrile illness. The presence of a minor infection is not a contraindication.
- A protective immune response may not be elicited by vaccines.
- In immunocompromised individuals, vaccination may not result in an appropriate protective antibody response.
- Vaccines should not be administered to participants with known hypersensitivity to any component of the vaccine or to participants having shown signs of hypersensitivity after previous administration. Appropriate medical treatment and supervision must always be readily available in case of a rare anaphylactic event following administration of the vaccine (see also anaphylaxis risk for altSonflex1-2-3 as described above).
- Anxiety-related reactions, including vasovagal reactions (syncope), hyperventilation or stress-related reactions may occur in association with vaccination as a psychogenic response to the needle injection; however, participants will be closely monitored for 60 minutes after vaccination.
- Vaccination should be administered with caution to participants with thrombocytopenia or a bleeding disorder since bleeding may occur following an intramuscular administration to these participants.

Risk mitigation strategy

The mitigation strategy that will be applied in this study for the risks linked to the study vaccines are collection, monitoring and reporting of AEs following vaccination. These vaccines should be administered by trained health care professionals who should follow the labelled instructions for vaccine preparation and administration. It is Good Clinical Practice (GCP) to perform a physical examination prior to vaccination and for medical treatment to be readily available after vaccination in case of severe or serious adverse reactions.

2.3.2. Benefit assessment

Participants or participants' parents can gain medical advice about their own or their children's health status through the medical evaluations and laboratory assessments associated with the study.

Vaccination with the investigational vaccine may provide protection against *S. sonnei* and *S. flexneri* bacteria. Vaccines administered as control and not routinely given will be provided free of charge and can provide protection against additional diseases (meningitis, typhoid, diphtheria, tetanus, pertussis***, hepatitis B, poliomyelitis, and Haemophilus influenzae type b***). Additionally, children and infants who were not administered MENVEO as a control vaccine during the study will be offered the opportunity to receive a meningitis vaccine at the end of the study.

The participants, by participating in the study, will contribute to the process of developing therapies in an area of unmet need. They may also help provide valuable data, about the tolerability and immunogenicity of the investigational vaccine and about its potential ability to provide clinical protection against shigellosis.

2.3.3. Overall Benefit: Risk conclusion

Considering the measures to minimise risk to participants enrolled in this study, the potential risks associated with the GVGH altSonflex1-2-3 vaccine are justified by the potential benefits that may be provided to the study participants.

The control vaccines administered to participants in this study as active comparators are widely used, and therefore have demonstrated medical benefit in the prevention of invasive meningococcal disease caused by *Neisseria meningitidis* groups A, C, W, Y (MENVEO), diphtheria/tetanus/pertussis ***(BOOSTRIX), diphtheria/tetanus/pertussis/hepatitis B/polyomelitis (inactivated)/Haemophilus influenzae type b (INFANRIX HEXA)***, or typhoid fever caused by *Salmonella typhi* (TYPHIM VI).

3. OBJECTIVES AND ENDPOINTS

Table 6 Study objectives and endpoints

Objectives	Endpoints
Primary	
• To identify the preferred dose of each component of the altSonflex1-2-3 vaccine (low, medium, or high) for infants 9 months of age in Africa (Stage 2).	• Anti-serotype specific *Shigella* lipopolysaccharide (LPS)/OAg* serum immunoglobulin G (IgG) geometric mean concentrations (GMC), as measured by GVGH enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the third vaccination (Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the safety and reactogenicity of the altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with unsolicited adverse events (AEs) during 28 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with serious adverse events (SAEs) during their entire study participation period (from Day 1 to Day113/Day 197). • Number of adults 18 to 50 years of age in Europe with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92/Day 176).

Objectives	Endpoints
	• Number of adults 18 to 50 years of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of adults 18 to 50 years of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of children 24 to 59 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of children 24 to 59 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of infants 9 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 281). • Number of infants 9 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8, Day 92, and Day 260).
Secondary	
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Europe (Stage 1).	• Anti-serotype specific *Shigella* LPS/O-Antigen (OAg)* serum IgG geometric mean concentrations (GMCs), in adults 18 to 50 years of age in Europe, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197).

Objectives	Endpoints
	**S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Africa (Stage 2).	• Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in adults 18 to 50 years of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti- serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in children 24 to 59 months of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in infants 9 months of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
To further characterise the immunogenicity profile of the *S. sonnei* component of altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).

Objectives	Endpoints
	*This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. **This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.
• To evaluate seroresponse with the altSonflex1-2-3 vaccine after each vaccination in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 14 days after first vaccination (Day 15) and at 28 days after each vaccination (Day 29 and Day 113/Day 197), compared to baseline (Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Africa showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85). • Number of children 24 to 59 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85). • Number of infants 9 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29, Day 113, and Day 281), compared to baseline (Day 1, Day 85, and Day 253). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity of co-administered MR-VAC in infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Anti-measles IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Anti-rubella IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-measles IgG concentrations of ≥150 mIU/mL* and ≥ 200 mIU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-rubella IgG concentrations of ≥4 IU/mL* and ≥10 IU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). *This threshold is based on the vaccine correlate of protection and World Health Organization guidelines.

Objectives	Endpoints
Tertiary	
• To evaluate the immune response induced by the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1), subsets of adults, children, and infants in the safety cohorts, and all infants in the dose-finding cohort, in Africa (Stage 2), using the serum bactericidal assay (SBA).	• Anti-serotype specific* serum bactericidal antibody GMTs in 25% of adults in Europe 18 to 50 years of age, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of adults in Africa 18 to 50 years of age, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113) • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of children 24 to 59 months of age before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113) • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of infants 9 months of age in the safety cohort, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Anti-serotype specific* serum bactericidal antibody GMTs in all infants 9 months of age in the dose-finding cohort, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281) **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and a subset of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Antibody features (subclassing, affinity, avidity) and functional properties (induction of cellular phagocytosis and cellular cytotoxicity, natural killer [NK] cell activation, eosinophil and basophil degranulation, complement deposition, neutrophil activation/phagocytosis, dendritic cell phagocytosis, mucin binding, glycosylation, and Fc receptor alteration), as measured by systems serology analysis. • Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous *Shigella* serotypes, as measured by SBA. • Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine homologous and heterologous *Shigella* serotypes, as measured by opsonophagocytosis assay.
• To measure natural exposure to *Shigella* in Stage 2 ***infant*** participants.	• Anti-*Shigella*-conserved protein IgG, as measured by anti-protein ELISA.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ***adults, children, and infant safety cohorts, in Africa (Stage 2)***.	• Anti-serotype specific *Shigella* LPS* serum IgG GMTs, in adults 18 to 50 years of age in ***Africa***, as measured by reference ELISA, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in children 24 to 59 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in infants 9 months of age in the safety cohorts in Africa, as measured by reference ELISA, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).***

Objectives	Endpoints
	• Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:800** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:1600*** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113).*** • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested. **This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. ***This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

4. STUDY DESIGN

4.1. Overall design

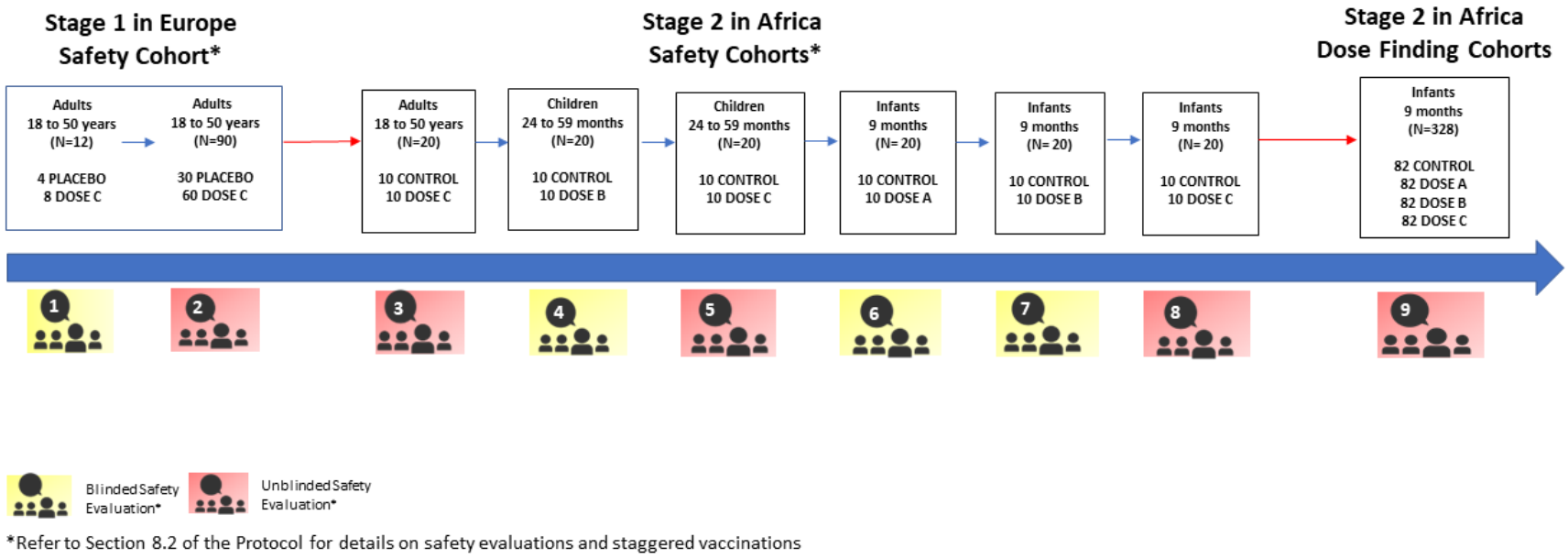
This is a Phase 1/2 observer-blind, controlled, self-contained, randomised, multi-country, staged, age-de-escalation study. An electronic Case Report Form (eCRF) and a paper Diary (pDiary) will be used for data collection, which will include biological sampling (blood) and safety follow-ups.

The study has 2 adult safety cohorts (18 to 50 years age in Europe and Africa), 2 child safety cohorts (24 to 59 months of age in Africa), 3 infant safety cohorts (9 months of age in Africa), and 1 infant dose-finding cohort (9 months of age in Africa). Figure 1 shows a visual representation of the study design. The study has a total of 20 study intervention groups as shown in Table 7, Table 8, Table 9, and Table 10 for Stage 1 adults, Stage 2 adults, Stage 2 children, and Stage 2 infants, respectively.

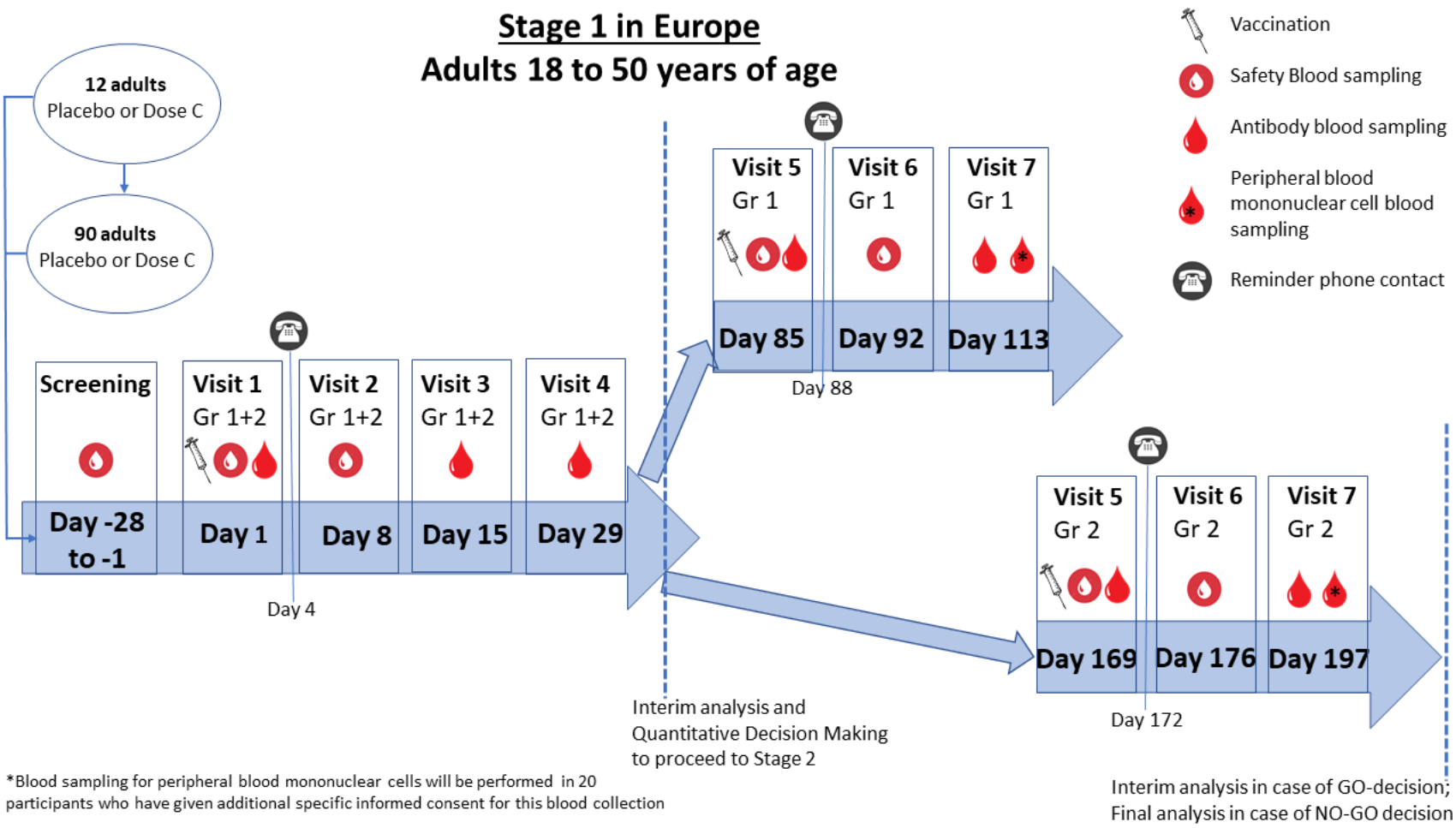
The study design, the enrolment, and the monitoring procedures will follow GSK safety standards for FTIH clinical studies (refer to Sections 8.2.2 and 10.1.5 for details on staggered vaccination, safety monitoring, and holding rules). For the study to proceed to Stage 2 in Africa, in addition to a favourable safety evaluation, a comprehensive review of Stage 1 data will also be performed with the support of a Quantitative Decision Making (QDM) approach (refer to Section 9.2.1). In case, a No-Go decision is made, the study will be discontinued early and complete at the end of Stage 1.

The primary completion date will be at Visit 9 (Day 281) in the Stage 2 infant dose-finding cohort.

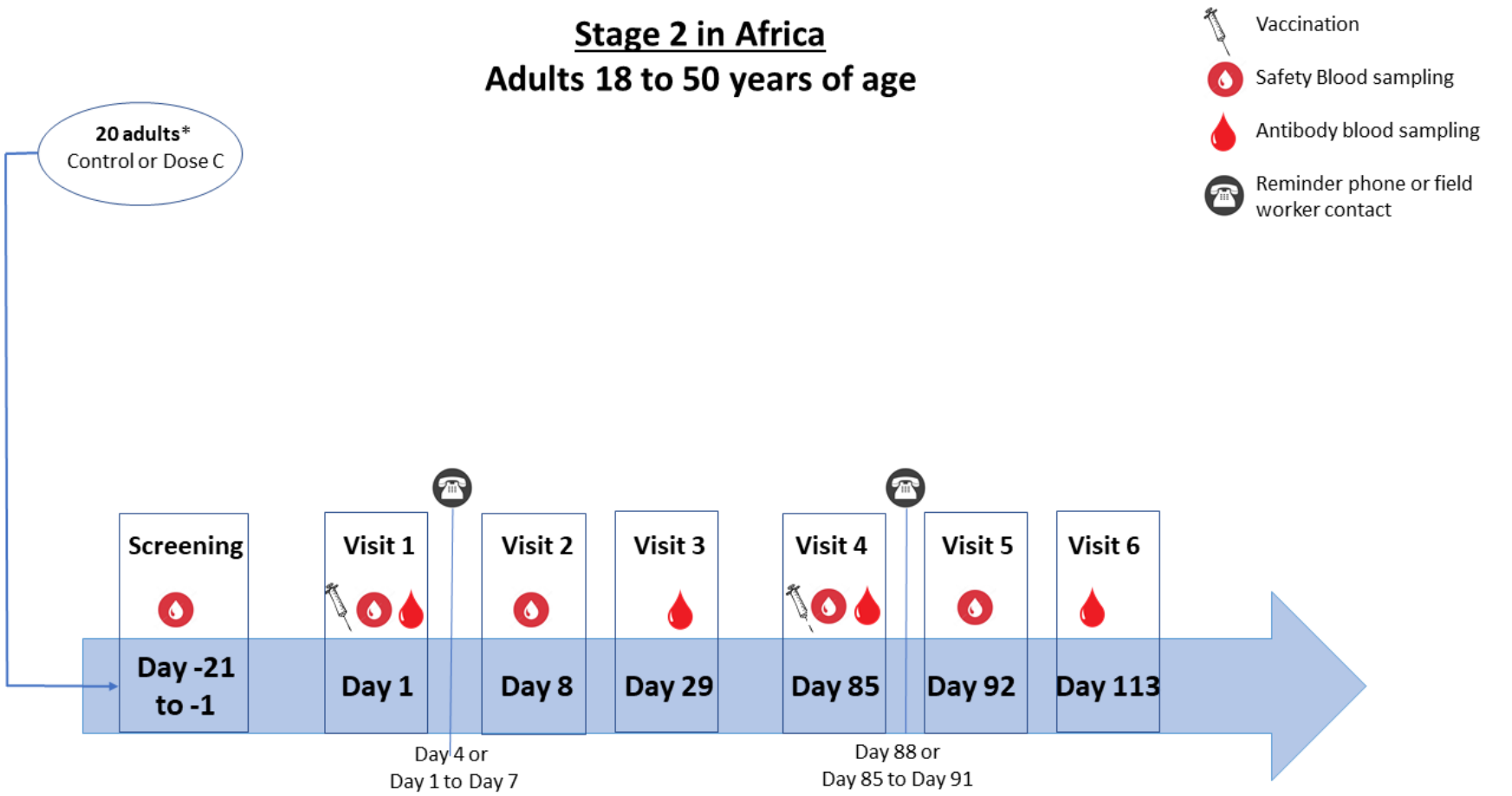
Figure 1 Study design overview



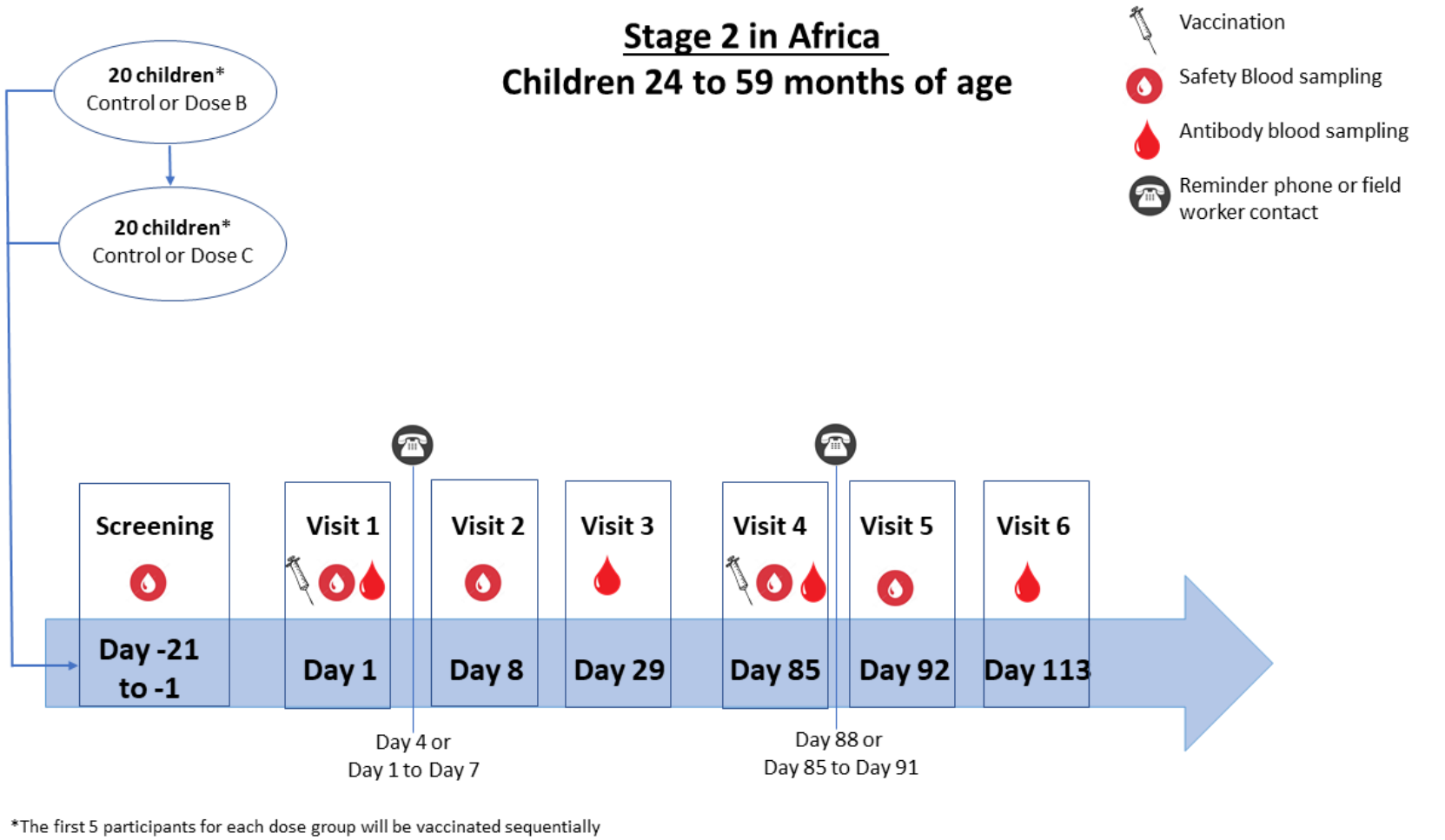
*Refer to Section 8.2 of the Protocol for details on safety evaluations and staggered vaccinations



*Blood sampling for peripheral blood mononuclear cells will be performed in 20 participants who have given additional specific informed consent for this blood collection



*The first 5 participants will be vaccinated sequentially



*The first 5 participants for each dose group will be vaccinated sequentially

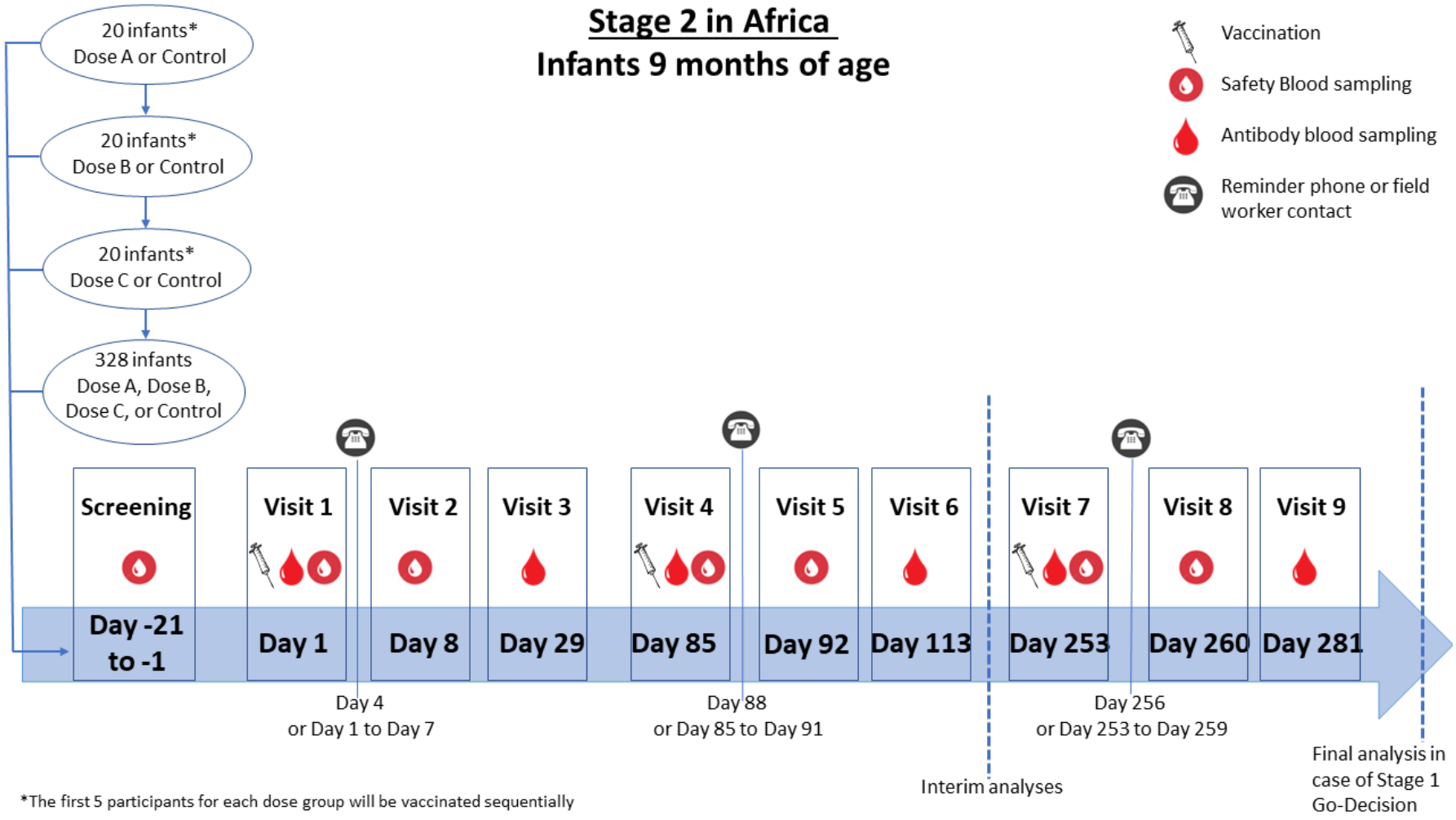

Stage 2 in Africa
Infants 9 months of age
20 infants*
Dose A or Control
20 infants*
Dose B or Control
20 infants*
Dose C or Control
328 infants
Dose A, Dose B,
Dose C, or Control
Vaccination
Safety Blood sampling
Antibody blood sampling
Reminder phone or field worker contact
Screening
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
Visit 7
Visit 8
Visit 9
Day -21 to -1
Day 1
Day 8
Day 29
Day 85
Day 92
Day 113
Day 253
Day 260
Day 281
Day 4
or Day 1 to Day 7
Day 88
or Day 85 to Day 91
Day 256
or Day 253 to Day 259
Interim analyses
Final analysis in case of Stage 1 Go-Decision
*The first 5 participants for each dose group will be vaccinated sequentially

Table 7 Study groups, intervention and blinding in Stage 1

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 7 (observer-blind)
ST1_Adults_Placebo_GR1	17	18 to 50 years (Adults)	altSonflex Placebo (Day 1 and Day 85)	●
ST1_Adults_Dose C_GR1	34	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●
ST1_Adults_Placebo_GR2	17	18 to 50 years (Adults)	altSonflex Placebo (Day 1 and Day 169)	●
ST1_Adults_Dose C_GR2	34	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 169)	●

Table 8 Study groups, intervention and blinding in adults in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 6 (observer-blind)
ST2_Adults_Control C	10	18 to 50 years (Adults)	MENVEO (Day 1), BOOSTRIX (Day 85)	●
ST2_Adults_Dose C	10	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●

Table 9 Study groups, intervention and blinding in Children in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 6 (observer-blind)
ST2_Children_Control B	10	24 to 59 months (Children)	MENVEO (Day 1), TYPHIM VI (Day 85)	●
ST2_Children_Dose B	10	24 to 59 months (Children)	Dose B altSonflex1-2-3 (Day 1 and Day 85)	●
ST2_Children_Control C	10	24 to 59 months (Children)	MENVEO (Day 1), TYPHIM VI (Day 85)	●
ST2_Children_Dose C	10	24 to 59 months (Children)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●

Table 10 Study groups, intervention and blinding in Infants in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 9 (observer-blind)
ST2_Infants_Control A_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose A_Safety	10	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	•
ST2_Infants_Control B_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose B_Safety	10	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	•
ST2_Infants_Control C_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose C_Safety	10	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	•
ST2_Infants_Control_Dose find	82	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 1 and Day 253), ***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose A_Dose find	82	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	•
ST2_Infants_Dose B_Dose find	82	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	•
ST2_Infants_Dose C_Dose find	82	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	•

4.2. Scientific rationale for study design

75% of deaths from shigellosis in children occur in Africa and South-East Asia. Both *S. sonnei* and *S. flexneri* are present in LMIC with ~24% being attributable to *S. sonnei*, ~20% to *S. flexneri* 2a, and ~10% each to *S. flexneri* 1b, 2b, 3a, and 6 [Livio, 2014]. AltSonflex1-2-3 would be able to target an important part of the responsible serotypes that affect LMIC.

4.2.1. Rationale for choice of vaccination route and schedule

- For the *S. sonnei* GMMA vaccine, the IM route of administration was the most immunogenic among the 3 vaccine administration routes tested (ID and IN were also tested) in a previous clinical study (NCT02017899 and NCT02034500).
- There was no evidence of clinically significant increase in reactogenicity with subsequent vaccine administrations in 1790GAHB Phase I and II studies, which used vaccination schedules of: 3 doses (up to 6/100 µg OAg/GMMA proteins of S. *sonnei* 1790 GAHB), 1 month apart (NCT02017899, in Europe) and 2 doses (up to 6/100 µg OAg/GMMA proteins of S. *sonnei* 1790 GAHB), 1 month apart (NCT02676895, in Kenya). Thus, a 3- or 6-month interval between vaccinations is unlikely to result in any safety concern.
- An extension study of the Phase I study was also performed to evaluate the safety and immunogenicity of a booster vaccination **(**NCT03089879**)**, which was essentially a fourth vaccination. The booster vaccination was well tolerated and the safety profile of the fourth 1790GAHB dose in previously primed participants was similar to that of a first vaccination given to vaccine naïve participants in the same trial.
- The altSonflex1-2-3 formulation is similar to the 1790GAHB formulation, previously tested in several clinical studies and with a similar safety profile in preclinical studies.
- In the previous CHIM study (NCT03527173), 2 vaccinations with a short 4-week interval were deemed to provide insufficient immunogenicity. A 3 month or 6-month interval between vaccinations, is hypothesised to be more immunogenic.
- Infants are likely to require more vaccinations to achieve adequate immune responses compared to older children and adults. A previous *Shigella* vaccine glycoconjugate failed to protect children when 2 vaccinations were administered [Passwell, 2010]. Additionally, other *Shigella* vaccines in development are using at least 3 vaccinations in infants (NCT04602975 and NCT04056117).

Based on all the above considerations, the schedule and route of administration of the candidate altSonflex1-2-3 is expected to show an acceptable safety profile in humans, comparable to the one of the *S. sonnei* 1790GAHB vaccine, whose safety has already been evaluated in 5 clinical studies.

4.2.2. Rationale for choice of study population

Vaccination of a paediatric population for *Shigella* represents an area of unmet need. Infants 9 months of age are the target population since this age group is particularly vulnerable to consequences of *Shigella* infections, particularly in LMIC. The exact age is chosen based on the epidemiology of shigellosis and it matches the recommended age for measles vaccination as per the EPI programme.

The choice of study populations involves a gradual transition to increased risk and population vulnerability, which depends on positive safety outcomes. The vaccine will first be tested in adults in Europe, a population not endemic for shigellosis and a setting considered as low-risk and low vulnerability, with high access to healthcare. The age range 18 to 50 years of age chosen for adults is consistent with previous *Shigella* studies performed by GVGH and also other studies investigating *Shigella* vaccines. Upon demonstrating positive safety and immunogenicity outcomes, the candidate vaccine will be tested in a population endemic for shigellosis, in adults in Africa. Again, only after positive safety outcomes, the candidate vaccine will be tested in children and finally in infants in Africa.

4.2.3. Rationale for choice of control vaccines

The rationales for choice of the control vaccines are:

For the adults in Africa, MENVEO AND BOOSTRIX have been used as comparators in a previous study conducted in Kenya (NCT02676895) during the development of the *S. sonnei* 1790GAHB vaccine. MENVEO is indicated for active immunisation to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y and W-135. The vaccine contains no preservative or adjuvant. Meningitis being prevalent in Kenya, providing a dose of MENVEO will provide some benefits to the participants. BOOSTRIX provides protection against tetanus, diphtheria, and pertussis. It can be administered as a booster in this age population.

For the children in Africa, MENVEO and TYPHIM VI will be administered because the timing of administration matches their recommended administrations. Meningitis vaccination is not routinely given as part of the EPI in Kenya but is offered privately at 9 months of age. The typhoid vaccine is also offered privately starting at 2 years of age.

For the infants in Africa, MENVEO and ***INFANRIX HEXA*** will be administered because the timing of administration matches their recommended administrations.

Active comparator vaccines are being used in Stage 2 in Africa to provide some benefit to study participants randomised to the control groups of the study. The vaccines selected would not be routinely available to the population in the study geographical region outside the clinical study setting.

4.2.4. Rationale for use of placebo

Since there is no widely available vaccine against shigellosis, the immunogenicity and safety profiles of the altSonflex1-2-3 vaccine will be evaluated and characterised against a placebo, altSonflex Placebo, in Stage 1. The population who will receive altSonflex Placebo in Stage 1 is not in an endemic area for shigellosis.

4.3. Justification for dose

- In preclinical studies, the dose levels of the altSonflex1-2-3 formulation used in this study were able to induce anti-LPS/OAg Ig with bactericidal activities. Addition of Alhydrogel to GMMA is not known to enhance antigenicity but attenuates the rise of body temperature in the first hours after vaccination.
- A GMMA vaccine has been previously used as a single component *S. sonnei* formulation at a dose of up to 6/100 µg OAg/protein with an acceptable safety profile in studies in Europe and Africa [Launay, 2017; Obiero, 2017]. The starting dose of OAg (15 µg) in adults is selected to be higher than the dose used in the previous CHIM study (NCT03527173), which showed negative results for efficacy with 1.5/25 µg OAg/protein and insufficient immunogenicity. An O-Antigen dose of 25 µg as a glycoconjugate vaccine showed high immunogenicity and 74% protective efficacy against shigellosis in adults [Cohen, 1997]. Also, a synthetic *Shigella* conjugate investigational vaccine, SF2a-TT15, containing 10 mg of O-Antigen induced a much stronger immune response than one containing 2 mg [Cohen, 2021], supporting the rationale that an O-Antigen content much higher than 1.5 mg is needed for the GMMA vaccine as well.
- The OAg is not thought to contribute to reactogenicity of the vaccine. Lipid A is one of the reactogenic components of GMMA and therefore modified to reduce its reactogenicity. The total amount of modified Lipid A in the highest dose of altSonflex1-2-3 to be used in this study is lower than in the highest doses of the *S. sonnei* vaccine used in Phase I and II studies.
- Lower starting doses will be used for the children and infant groups to mitigate any potential risks related to dose increases.
- Previous toxicology studies with 1790GMMA and Sonflex-1-2-3 showed that doses used were well tolerated and did not show evidence of systemic toxicity. Results from a toxicology study with the highest dose of altSonflex1-2-3 have shown a similar safety profile.

4.4. End of Study definition

A participant is considered to have completed the study if he/she returns for the last visit or is available for the last scheduled procedure as described in the protocol.

End of Study (EoS):

Date of 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 Last Subject Last Visit (LSLV).

5. STUDY POPULATION

Adherence to the inclusion and exclusion criteria specified in the protocol is essential. Deviations from these criteria are not allowed because they can jeopardise the scientific integrity, regulatory acceptability of the study or safety of the participant.

5.1. Inclusion criteria

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

- Participants and/or participants' parent(s)/legally acceptable representative(s) LAR(s), 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 or witnessed/thumb printed informed consent obtained from the participant/parent(s)/LAR(s) of the participant prior to performance of any study specific procedure.
- Healthy participants as established by medical history**,** clinical examination, and laboratory assessment.
- Participants satisfying all screening requirements.
- Participants seronegative for hepatitis B, and hepatitis C.
- Participants negative for human leukocyte antigen B27 (HLA-B27).

Adult participants must satisfy ALL the following criteria at study entry:

- A male or female between, and including, 18 and 50 years of age at the time of the first study intervention administration.
- Female participants 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. Refer to Section 10.4.1 for definitions of women of childbearing potential, menarche, and menopause.
- Female participants of childbearing potential may be enrolled in the study if the participant:
  - has practiced adequate contraception for 1 month prior to study intervention administration, and
  - has a negative pregnancy test on the day of study intervention administration, and
  - has agreed to continue adequate contraception during the entire treatment period and for 1 month after completion of the study intervention administration series.
- Participants seronegative for human immunodeficiency virus (HIV)

Child participants must satisfy ALL the following criteria at study entry:

- A male or female between, and including, 24 and 59 months of age at the time of first vaccination
- Normal nutritional Z score (-2 standard deviation or greater)
- Previously completed routine childhood vaccinations to the best knowledge of the participant's parent(s)/LAR(s)
- Born after gestation period of ≥37 weeks
- Participants seronegative for HIV

Infant participants must satisfy ALL the following criteria at study entry:

- A male or female 9 months of age at the time of first vaccination.
- Normal nutritional Z score (-2 standard deviations or greater).
- Previously completed routine childhood vaccinations to the best knowledge of the participant's parent(s)/LAR(s).
- Born after a gestation period of ≥37 weeks
- Participants negative for HIV as confirmed by deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) testing

5.2. Exclusion criteria

The following criteria should be checked at the time of study entry. The potential participant MUST NOT be included in the study if ANY exclusion criterion applies:

5.2.1. Medical conditions

All participants:

- Known exposure to *Shigella* during lifetime of the participant as confirmed during interview with the participant or documented by patient records (e.g., history of microbiologically-confirmed *Shigella* infection), recent travel* (within 2 years) to a country where *Shigella* or other enteric infections are endemic, or recent occupation* (within 3 years) involving *Shigella* species

  *Exclusion due to travel or occupation is applicable only to Adults 18 to 50 years of age in Europe (Stage 1). Refer to Section 8.2.1 for additional instructions and Section 10.9 for the list of countries considered highly endemic for *Shigella*.
- Progressive, unstable, or uncontrolled clinical conditions.
- History (known or suspected) of any reaction or hypersensitivity likely to be exacerbated by any component of the study vaccine.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).

- Hypersensitivity, including allergy, to medicinal products or medical equipment whose use is foreseen in this study.
- Clinical conditions representing a contraindication to IM vaccination and blood draws.
- Any behavioural or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the participant's ability to participate in the study.
- Acute disease and/or fever (defined as temperature ≥38.0°C) at the time of enrolment*.

  *The participant can still be enrolled into the study at a time when the acute disease and/or fever has resolved.
- Any clinically significant* haematological and/or biochemical (see safety screening laboratory assays in Table 27) laboratory abnormality.

  * The investigator should use his/her clinical judgement to decide which abnormalities are clinically significant.
- Confirmed positive COVID-19 test during the period starting 30 days before the first administration of study vaccines (Day -30 to Day 1)
- Any other clinical condition that, in the opinion of the investigator, might pose additional risk to the participant due to participation in the study.

Adults 18 to 50 years of age:

- Acute* or chronic illness, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests.
  *Participants with a minor illness (such as mild diarrhoea or mild upper respiratory infection) without fever may be enrolled at the discretion of the investigator.

Children 24 to 59 months of age:

- Acute or chronic clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests.

Infants 9 months of age:

- Acute or chronic clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests.

5.2.2. Prior/Concomitant therapy

All participants:

- Administration of long-acting immune-modifying drugs at any time during the study period (e.g., infliximab).
- Prior receipt of an experimental *Shigella* vaccine or live *Shigella* challenge.
- Use of any investigational or non-registered product (drug, vaccine, or medical device)* other than the study vaccine during the period starting 30 days before the first dose of study intervention (Day -30 to Day 1), or planned use during the study period.

  *Use of herbs and traditional treatments is not considered an exclusion criterion.
- A vaccine not foreseen* by the Study Protocol administered during the period starting at -21 days before the first dose (-28 days in the case of live vaccines) and ending after the last dose of study intervention administration**.

  *Vaccines allowed by the protocol include flu vaccines and COVID-19 vaccines in all participants, and EPI vaccines in children and infants. The recommended time intervals for administration of these vaccines is at least 7 days before or 7 days after (at least 14 days before or 14 days after in case of live vaccines) each dose of study intervention administration. In case these allowed vaccines are administered outside the aforementioned interval during the study, the participant should continue to receive the subsequent study intervention administration as per planned schedule.

  **In case emergency mass vaccination for an unforeseen public health threat (e.g., a pandemic) is recommended and/or organised by public health authorities outside the routine immunisation programme, the time period described above can be reduced if, necessary for that vaccine, provided it is used according to the local governmental recommendations and that the sponsor is notified accordingly. When regulations allow, the recommended time intervals for administration of these vaccines is at least 7 days before or 7 days after (at least 14 days before or 14 days after in case of live vaccines) each dose of study intervention administration.

Adults 18 to 50 years of age:

- Administration of immunoglobulins and/or any blood products or plasma derivatives, or bone marrow transplantation**,** during the period starting 3 months before the first dose of study vaccine or planned administration during the study period.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 3 months prior to the first vaccine study intervention. For corticosteroids, this will mean prednisone equivalent ≥20 mg/day for adult participants. Inhaled and topical steroids are allowed.

Children 24 to 59 months of age:

- Administration of immunoglobulins and/or any blood products or plasma derivatives**,** or bone marrow transplantation, during the period starting 3 months before the first dose of study vaccine or planned administration during the study period.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 3 months prior to the first vaccine dose. For corticosteroids, this will mean prednisone ≥0.5 mg/kg/day or 20 mg/day whichever is the maximum dose for paediatric participants. Inhaled and topical steroids are allowed.

Infants 9 months of age:

- Administration of immunoglobulins and/or any blood products or plasma derivatives**,** or bone marrow transplantation, from birth or planned administration during the study period.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 3 months prior to the first vaccine dose. For corticosteroids, this will mean prednisone ≥0.5 mg/kg/day or 20 mg/day whichever is the maximum dose for paediatric participants. Inhaled and topical steroids are allowed.

5.2.3. Prior/Concurrent clinical study experience

All participants:

- Concurrently participating in another clinical study, at any time during the study period, in which the participant has been or will be exposed to an investigational or a non-investigational intervention (drug or invasive medical device).

5.2.4. Other exclusions

All participants:

- Any study personnel or immediate dependents, family, or household member.

Adults 18 to 50 years of age in Stage 1 (Europe) and Stage 2 (Africa):

- Pregnant or lactating female.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- History of or current chronic* alcohol consumption and/or drug abuse.

*Chronic alcohol consumption is defined as one or more of the following: a) a prolonged period of frequent and heavy alcohol use, b) the inability to control drinking once it has begun, c) physical dependence manifested by withdrawal symptoms when the individual stops using alcohol, d) tolerance or the need to use increasing amounts of alcohol to achieve the same effects, e) and a variety of social and/or legal problems arising from alcohol use.

Children 24 to 59 months of age and infants 9 months of age:

- Child in care.

Please refer to the Section 10.10.2 for the definition of child in care.

5.3. Lifestyle considerations

Not applicable

5.4. Screen failures

A screen failure is an individual who consents to participate in this this study but is not entered in the study (was not randomised, did not receive study intervention, and did not undergo a post-screening study procedure).

The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

5.5. Criteria for temporarily delaying enrolment and study intervention administration

Enrolment/study intervention administration may be postponed within the permitted time interval until transient conditions cited below are resolved:

- Participants with haematological/biochemical values out of normal range which are expected to be temporary may be rescreened/re-vaccinated at a later date within the allowed time interval.
- Acute disease and/or fever at the time of enrolment and/or study intervention administration. Refer to the SoA for definition of fever and preferred location for measuring temperature in this study.
- Participants with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever may be enrolled and/or dosed at the discretion of the investigator.
- Use of antipyretics and/or analgesics and/or antibiotics within 3 days prior to study intervention administration.
- Participants with a positive COVID-19 test before a planned study intervention administration will be allowed to receive the study intervention only if at least 14 days have passed since the first positive test.

6. STUDY INTERVENTION AND CONCOMITANT THERAPY

Refer to the Glossary of terms for the definition of study intervention.

6.1. Study interventions administered

Table 11 shows the study interventions that will be administered during the study.

The candidate vaccine, altSonflex1-2-3, will be administered intramuscularly by unblinded study site staff and contains 15 µg (Dose C), 7.5 µg (Dose B), or 3.75 µg (Dose A) of OAg for each antigen component (refer to the Study Procedures Manual [SPM] for additional details). The vaccine is available as a suspension of GMMA bound to Alhydrogel and does not contain any preservative. The placebo intervention (altSonflex Placebo) contains all components except antigen and will be used to assess the safety of the vaccine in Stage 1. Table 11 shows formulation details of all study interventions to be administered in the study.

All study participants must be observed closely for at least 60 minutes after the administration of the study interventions. Appropriate medical treatment must be readily available during the observation period in case of anaphylaxis, syncope. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

Table 11 Study interventions administered

Study intervention name	AltSonflex1-2-3 Dose A (diluted from Dose C)		AltSonflex1-2-3 Dose B (diluted from Dose C)		AltSonflex 1-2-3 Dose C	altSonflex Placebo	***MENVEO***		***BOOSTRIX***	***INFANRIX HEXA***		***TYPHIMVI***	***MR-VAC***	
Study intervention formulation	*Shigella sonnei* GMMA adsorbed on $Al(OH)_3$(3.75 µg OAg); *Shigella flexneri 1b* GMMA adsorbed on $Al(OH)_3$ (3.75 µg OAg); *Shigella flexneri 2a* GMMA adsorbed on $Al(OH)_3$ (3.75 µg OAg); *Shigella flexneri 3a* GMMA adsorbed on $Al(OH)_3$ (3.75 µg OAg); $Al(OH)_3$ suspended	$Al(OH)_3$ suspended in buffered saline (q.s. 0.375 mL)	*Shigella sonnei* GMMA adsorbed on $Al(OH)_3$ (7.5 µg OAg); *Shigella flexneri 1b* GMMA adsorbed on $Al(OH)_3$ (7.5 µg OAg); *Shigella flexneri 2a* GMMA adsorbed on $Al(OH)_3$ (7.5 µg OAg); *Shigella*	$Al(OH)_3$ suspended in buffered saline (q.s. 0.25 mL)	*Shigella sonnei* GMMA adsorbed on $Al(OH)_3$ (15 µg OAg); *Shigella flexneri 1b* GMMA adsorbed on $Al(OH)_3$ (15 µg OAg); *Shigella flexneri 2a* GMMA adsorbed on $Al(OH)_3$ (15 µg OAg); *Shigella flexneri 3a* GMMA adsorbed on $Al(OH)_3$ (15 µg	$Al(OH)_3$ suspended in buffered saline (q.s. 0.5 mL)	MenA(10 µg)-CRM_{197} (16.7-33.3 µg)	MenC(5 µg)-CRM_{197} (7.1-12.5 µg); MenW-135(5 µg)-CRM_{197}(3.3-8.3 µg); MenY(5 µg)-CRM_{197} (5.6-10 µg); Water for injections q.s. 0.5 mL	DT (≥ 2 IU) adsorbed on aluminium hydroxide and aluminium phosphate; TT (≥ 20 IU) adsorbed on aluminium hydroxide and aluminium phosphate; PT (8 µg) adsorbed on aluminium hydroxide and	***DT[1] (≥ 30 IU); TT[1] (≥ 40 IU); PT (25 µg)[1]; FHA (25 µg)[1]; PRN (8 µg)[1]; HBsAg (10 µg)[2]; IPV1 (Mahoney strain) (40 DAgU); IPV2 (MEF-1 strain) (8 DAgU); IPV3 (Saukett strain) (32 DAgU); [1]adsorbed on $Al(OH)_3$ and [2]adsorbed on $AlPO_4$ (0.7 mg Al^{3+}); Water for injections***	***Hib (10 µg)-TT (~25 µg) adsorbed on $AlPO_4$; $AlPO_4$***	Vi capsular polysaccharide of Salmonella typhi (Ty2 strain) (25 µg); Water for Injections q.s. 0.5 mL	Measles Virus Edmonston strain (live, attenuated) (≥ 1000 $CCID_{50}$); Rubella Virus Wistar RA 27/3 strain (live, attenuated) (≥ 1000 $CCID_{50}$)	Water for injections (0.5 mL)

Study intervention name	AltSonflex1-2-3 Dose A (diluted from Dose C)		AltSonflex1-2-3 Dose B (diluted from Dose C)		AltSonflex 1-2-3 Dose C	altSonflex Placebo	***MENVEO***		***BOOSTRIX***	***INFANRIX HEXA***		***TYPHIMVI***	***MR-VAC***	
	in buffered saline (q.s. 0.125 mL)		*flexneri 3a* GMMA adsorbed on $Al(OH)_3$ (7.5 µg OAg); $Al(OH)_3$ suspended in buffered saline (q.s. 0.25 mL)		OAg); $Al(OH)_3$ suspended in buffered saline (q.s. 0.5 mL)				aluminium phosphate; FHA (8 µg) adsorbed on aluminium hydroxide and aluminium phosphate; PRN (2.5 µg) adsorbed on aluminium hydroxide and aluminium phosphate; Aluminium hydroxide (0.3 mg Al^{3+}); Aluminiu					

Study intervention name	AltSonflex1-2-3 Dose A (diluted from Dose C)		AltSonflex1-2-3 Dose B (diluted from Dose C)		AltSonflex 1-2-3 Dose C	altSonflex Placebo	***MENVEO***		***BOOSTRIX***	***INFANRIX HEXA***		***TYPHIMVI***	***MR-VAC***	
									m phosphate (0.2 mg Al^{3+}); Water for injections q.s. 0.5 mL					
Presentation	Vial, suspension for suspension for injection	Vial, Suspension for suspension for injection	Vial, suspension for suspension for injection	Vial, Suspension for suspension for injection	Vial, suspension for injection	Vial, suspension for injection	Vial, powder for solution for injection	Vial, solution for solution for injection	Syringe, suspension for injection	***Pre-filled syringe, suspension for suspension for injection***	***Vial, powder for suspension for injection***	Syringe, solution for injection	Powder for suspension for injection	Solution for suspension for injection
Type	***Investigational product***		***Investigational product***		***Investigational product***	***Placebo***	***Comparator***		***Comparator***	***Comparator***		***Comparator***	***Co-administered product***	
Product category	Biologic		Biologic		Biologic	Drug	Biologic		Combination Product	Combination Product		Combination product	Biologic	
Route of administration	intramuscular												Subcutaneous	
Location	Deltoid in adults and children, thigh in infants					Deltoid	Deltoid in adults and children, anterolateral thigh in infants		Deltoid	Thigh		Deltoid	Deltoid	
Directionality	Upper in adults and children, anterolateral in infants					Upper	Upper in adults and children,		Upper	Anterolateral		Upper	Upper	

Study intervention name	AltSonflex1-2-3 Dose A (diluted from Dose C)	AltSonflex1-2-3 Dose B (diluted from Dose C)	AltSonflex 1-2-3 Dose C	altSonflex Placebo	***MENVEO***	***BOOSTRIX***	***INFANRIX HEXA***	***TYPHIMVI***	***MR-VAC***
					anterolateral in infants				
Laterality *	Non-dominant								
Number of doses to be administered:	3 in infants	3 in infants, 2 in children	2 in adults and children, 3 in infants	2 in adults (Stage 1)	1 in adults (Stage 2), 1 in children, 2 in infants	1 in adults (Stage 2)	1 in infants	1 in children	2 in infants
Volume to be administered	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Packaging and labelling	Refer to the Study Procedures Manual (SPM) for further details								
Responsible for manufacture	Dilution at clinical site (Refer to the SPM for further details)		GSK					Sanofi Pasteur	Serum Institute of India

SPM=Study Procedures Manual

*The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the study intervention in the non-dominant arm, an injection in the dominant arm may be performed

6.2. Preparation/Handling/Storage/Accountability

The study interventions must be stored in a secured place within the temperature range specified on the study intervention's label. The storage temperature should be continuously monitored and recorded with a calibrated (if not validated) temperature monitoring device(s).

Only authorised study personnel should be allowed access to the study interventions. Storage conditions will be assessed by a sponsor study contact during pre-study activities. Refer to the SPM for more details on storage and handling of the study interventions.

6.3. Measures to minimise bias: randomisation and blinding

6.3.1. Participant identification

Participant identification numbers will be assigned sequentially to the participants who have consented to participate in the study, according to the range of participant identification numbers allocated to each study centre.

6.3.2. Randomisation to study intervention

The randomisation of supplies within blocks will be performed at GSK using Statistical Analysis System (Cary, NC, USA).

The study intervention numbers will be allocated by component.

6.3.3. Intervention allocation to the participant

The target will be to enrol approximately 550 eligible participants who will be randomly assigned to the different study groups, according to the study Stage:

- Stage 1 (Europe), safety cohort, 102 adults 18 to 50 years of age:
  - 102 participants will be randomly assigned in a 1:1:2:2 ratio to study groups "ST1_Adults_Placebo_GR1" (approximately 17 participants), "ST1_Adults_Placebo_GR2" (approximately 17 participants), "ST1_Adults_Dose C_GR1" (approximately 34 participants), and "ST1_Adults_Dose C_GR2" (approximately 34 participants), with a staggered enrolment approach; the first 12 participants will receive the first vaccination in a sequential manner, followed by the remaining 90 participants who will be vaccinated in parallel after a positive decision based on the safety evaluation of the first 12 participants.
- Stage 2 (Africa), safety cohort, 20 adults 18 to 50 years of age:
  - 20 participants will be randomly assigned in a 1:1 ratio to study groups "ST2_Adults_Control C groups" and "ST2_Adults_Dose C" (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.

- Stage 2 (Africa), safety cohort, 40 children 24 to 59 months of age:
  - 20 participants will be randomly assigned in a 1:1 ratio to study groups "ST2_Children_Control B" and "ST2_Children_Dose B" groups (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.
  - 20 participants will be randomly assigned in a 1:1 ratio to study groups "ST2_Children_Control C" and "ST2_Children_Dose C" groups (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.
- Stage 2 (Africa), safety cohort, 60 infants 9 months of age:
  - 20 participants will be randomly assigned in a 1:1 ratio to study groups "ST2_Infants_Control A_Safety" and "ST2_Infants_Dose A_Safety" groups (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.
  - 20 participants will be randomly assigned in a 1:1 ratio to "ST2_Infants_Control B_Safety" and "ST2_Infants_Dose B_Safety" groups (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.
  - 20 participants will be randomly assigned in a 1:1 ratio to study groups "ST2_Infants_Control C_Safety" and "ST2_Infants_Dose C_Safety" groups (approximately 10 participants in each group), with the first 5 participants administered the first vaccination in a sequential manner.
- Stage 2 (Africa), dose-finding cohort, 328 infants 9 months of age:
  - 328 participants will be randomly assigned in a 1:1:1:1 ratio to study groups "ST2_Infants_Control_Dose find", "ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose B_Dose find", and "ST2_Infants_Dose C_Dose find" (approximately 82 participants in each study group).

For additional details, refer to Section 8.2.2.1.

Allocation of the participant to a study group at the investigator site will be performed using a randomisation system on internet (source data base for internet randomisation, herein referred to as SBIR). When SBIR is not available, please refer to the SBIR user guide or the SPM for specific instructions.

After obtaining the signed and dated ICF from the participant/participant's parent(s)/LAR(s) and having checked the eligibility of the participant, the study staff in charge of the vaccine administration will access SBIR. Upon providing information about Stage, age, and cohort and the participant identification number, the randomisation system will determine the study group and will provide the study intervention number to be used for the first dose. The number of each administered study intervention must be recorded in the eCRF on the Vaccine Administration Form.

The randomisation algorithm will use a stratification procedure accounting for Stage (Stage 1 or Stage 2), age (adults, children, or infants), and cohort (safety or dose-finding) and a minimisation procedure accounting for study. Minimisation factors will have equal weight in the minimisation algorithm.

Once a participant identification number is allocated, the randomisation system will determine study group and will provide the study intervention number to be used for the first dose. The study intervention number(s) to be used for subsequent dosing will be provided by the same SBIR.

Refer to the SPM for additional information about the study intervention number allocation.

6.3.4. Intervention allocation for subsequent doses

For each dose subsequent to the first dose, the study staff in charge of the vaccine administration will access SBIR, provide the participant identification number, and the system will provide a study intervention number consistent with the allocated study group.

The number of each administered study intervention must be recorded in the eCRF on the Vaccine Administration Form.

6.3.5. Allocation of participants to assay subsets

- Stage 1 Adults Subset: 25% of participants will be randomly selected from each adult treatment study group in Stage 1 ("ST1_Adults_Dose C_GR1", "ST1_Adults_Dose C_GR2", "ST1_Adults_Placebo_GR1", and "ST1_Adults_Placebo_GR2"). For this randomly selected subset of participants, available samples taken at Days 1, 15, 29, 85/169, and 113/197 will be tested to fulfil tertiary objectives (SBA, ***SBA against a panel of vaccine heterologous Shigella serotypes,*** systems serology analysis, and opsonophagocytosis assay).
- Stage 2 Adults Subset: all participants from the adult altSonflex1-2-3 group in Stage 2 ("ST2_Adults_Dose C") and a random sample of 50% of participants from the adult control study group in Stage 2 ("ST2_Adults_Control C") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, and 113.
- Stage 2 Children Subset: all participants from children altSonflex1-2-3 groups in Stage 2 ("ST2_Children_Dose B" and "ST2_Children_Dose C") and a random sample of 25% participants from the children control study groups in Stage 2 ("ST2_Children_Control B" and "ST2_Children_Control C") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, and 113.

- Stage 2 Infants Safety Cohort Subset: all participants from the infant altSonflex1-2-3 safety cohort groups ("ST2_Infants_Dose A_Safety", "ST2_Infants_Dose B_Safety", and "ST2_Infants_Dose C_Safety") and a random sample of 33.33% participants from the infant safety cohort control study groups in Stage 2 ("ST2_Infants_Control A_Safety", "ST2_Infants_Control B_Safety", and "ST2_Infants_Control C_Safety") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, 113, and 281.
- Stage 2 Infants Dose-Finding Cohort Subset: a subset of participants from the infant dose-finding cohorts ("ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose B_Dose find", and "ST2_Infants_Dose C find") will be randomly selected. For this randomly selected subset of participants, systems serology analysis, SBA against a panel of vaccine heterologous *Shigella* serotypes, and opsonophagocytosis assay will be performed on available samples to fulfil tertiary objectives.

6.3.6. Blinding and unblinding

Data will be collected in an observer-blind manner. To do so, study interventions will be prepared and administered by qualified unblinded staff who will not participate in data collection, evaluation, review, or the entry of any study endpoint (i.e., reactogenicity, safety, efficacy).

In Stage 1, investigators will be unblinded to the vaccination schedule. However, investigators will remain blinded to each participant's assigned study intervention throughout the course of the study. Vaccine dilution and all possibly necessary additional activities involving vaccines (e.g., re-labelling of clinical study materials at the study site) will be performed by unblinded site staff according to instructions and training provided by GSK. The site staff responsible for these activities will be personnel who are respectively qualified according to applicable local laws and regulations. To maintain the blinding during the preparation and the administration of the vaccine(s), vaccines should be prepared and administered at a location different from the location where clinical assessment and evaluation is performed. The participant or participant's parent(s) or LAR(s) will be instructed not to look at the injection syringe while the study intervention is administered. The unblinded site staff will be also responsible for completing the Vaccine Administration Log and Vaccine Accountability Log to ensure final accountability. Refer to the SPM for further details on blinding procedures and management.

The laboratory in charge of sample testing will be blinded to the study intervention assignment. Codes will be used to link the participant and study to each sample. There will be no link between the study intervention group and the identity of the participant.

6.3.6.1. Emergency unblinding

Unblinding a participant's individual study intervention number should occur ONLY in case of a medical emergency when this information is essential for the clinical management or welfare of the participant.

The emergency unblinding process enables the investigator to have unrestricted, immediate, and direct access to the participant's individual study intervention via SBIR.

In case of emergency, the investigator can have unrestricted, immediate, and direct access to the participant's study intervention information via SBIR. The investigator may contact a GSK Helpdesk (refer to the Table 12) if he/she needs help to access participant's study intervention information (i.e., if the investigator is unable to access SBIR).

A physician other than the investigator (e.g., an emergency room physician) or participant/care giver/family member may also request emergency unblinding either via the investigator (preferred option) or via the GSK Helpdesk (back up option). The subject/participant card provides contact information for the investigator, his/her back up and GSK Helpdesk.

Table 12 Contact information for emergency unblinding

GSK Helpdesk Available 24/24 hours and 7/7 days
The Helpdesk is available by phone, fax, and email Phone: +32 2 656 68 04 (Kenya) and 00 800 4344 1111 (Belgium toll free) Fax: +32 2 401 25 75 Email: rix.ugrdehelpdesk@gsk.com

6.3.6.2. Unblinding prior to regulatory reporting of SAEs

GSK policy requires unblinding of any unexpected SAE which is attributable/suspected to be attributable to the study interventions, prior to regulatory reporting. GSK Global Safety (GS) is responsible for unblinding the study intervention assignment within the timeframes defined for expedited reporting of SAEs (refer to the Section 10.3.10.1).

In addition, GSK GS staff may unblind the intervention assignment for any participant with a Suspected Unexpected Serious Adverse Reaction (SUSAR) or a SAE that is fatal or life-threatening. For SAEs requiring expedited reporting to 1 or more regulatory agencies, a copy of the report containing participant's intervention assignment may be sent to investigators in accordance with local regulations and/or GSK policy.

6.4. Study intervention compliance

Participants will receive the study interventions directly from the investigator or designee, under medical supervision. The date of administration of each study intervention dose in the clinic will be recorded in the source documents.

6.5. Dose modification

Not applicable.

6.6. Continued access to study intervention after the end of the study

During the study conclusion visit, the investigator will ask each participant if they are interested in participating in a long-term study. If a participant is not interested in joining the long-term study, the reason for refusal will be documented, when available, in the participant's eCRF.

The investigator is encouraged to share the immunological assay results of non-responders with the study participants/participants' parent(s)/LAR(s).

It is the responsibility of the investigator (supported by GVGH) to determine the medical care of the non-responder as per local/regional practices, such as revaccination against measles and rubella in Stage 2.

After unblinding, the investigator will also be supported to offer vaccination with a single dose of a locally licensed 4-component meningitis vaccine to all children and infants who had not received MENVEO during the study.

6.7. Treatment of overdose

Not applicable.

6.8. Concomitant therapy

At each study visit, the investigator or his/her delegate should question the participant and/or the participant's parent(s)/LAR(s) about all medications/products taken, and vaccinations received by the participant.

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 of 28 days post-vaccination.
- Relevant medications/products such as vaccines, immunoglobulin, blood products, immunosuppressors, and immunomodulators, administered during the period starting from the administration of the first dose of the study vaccine/placebo and ending at the participant's last study visit/contact.
- Any concomitant vaccination administered in the period starting 30 days before the first dose of study vaccine and ending at the participant's last study visit/contact (Day -30 to Day 113/197 for adults 18 to 50 years of age in Stage 1, Day -30 to Day 113 for adults 18 to 50 years of age and children 24 to 59 months of age in Stage 2, Day -30 to Day 281 for infants 9 months of age in Stage 2).
- Prophylactic medication (i.e., medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

- All concomitant medication associated with an AEs, including vaccines/products, except vitamins and dietary supplements, administered after the first dose of study intervention (Day 1 to Day 113/197 for adults 18 to 50 years of age in Stage 1, Day 1 to Day 113 for children 24 to 59 months of age in Stage 2, Day 1 to Day 281 for infants 9 months of age in Stage 2).
- All concomitant medication leading to discontinuation of the study intervention or elimination from the analysis, including products/vaccines (refer to Sections 5.2.2 and 9.3.1 for further details).
- All concomitant medication which may explain/cause/be used to treat an SAE including vaccines/products, as defined in Sections 8.3.1 and 10.3.8.2. These must also be recorded in the Expedited Adverse Event report.

The GVGH Project Physician (PP) should be contacted if there are any questions regarding concomitant or prior therapy.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of study intervention

'Discontinuation' of study intervention refers to any participant who has not received all planned doses of study intervention. A participant who discontinued study intervention may continue other study procedures (e.g., safety or immunogenicity), planned in the Study Protocol at the discretion of the investigator.

The primary reason for premature discontinuation of the study intervention will be documented on the eCRF as follows:

- Adverse event requiring expedited reporting to GSK
- Unsolicited non-serious adverse event
- Solicited adverse event
- Not willing to be vaccinated
- Other (specify).

7.1.1. Contraindications to subsequent study intervention(s) administration

The eligibility for subsequent study intervention administration must be confirmed before administering any additional dose.

Participants who meet any of the criteria listed below or criteria listed in Sections 5.2.1 and 5.2.2 should not receive additional doses of study intervention. Such participants should be encouraged to continue other study procedures, at the investigators' discretion (Section 10.3.8.2). All relevant criteria for discontinuation of study intervention administration must be recorded in the eCRF.

- Participants who experience any SAE judged to be possibly or probably related to study intervention or non-study concomitant vaccine/product, including hypersensitivity reactions.
- Participants who develop any new condition which, in the opinion of the investigator, may pose additional risk to the participant if he/she continues to participate in the study.
- Participants who experienced any immediate allergic reaction, including anaphylaxis, within 24 hours after any previous study vaccination.
- Any condition that in the judgement of the investigator would make intramuscular injection unsafe.

7.2. Participant discontinuation/withdrawal from the study

A participant is considered to have withdrawn from the study if no new study procedure has been performed or no new information has been collected for him/her since the date of withdrawal/last contact.

From an analysis perspective, a study 'withdrawal' refers to any participant who did not return for the concluding visit planned in the protocol.

Investigators will attempt to contact participants who do not return for scheduled visits or follow-up.

All data and samples collected up to and including the date of withdrawal of/last contact with the participant will be included in the study analyses.

The primary reason for study withdrawal will be documented in the eCRF, based on the list below:

- Adverse events requiring expedited reporting to GSK (refer to Section 10.3.10 for details)
- Unsolicited non-serious adverse events
- Solicited adverse event
- Withdrawal by participant, not due to an adverse event*
- Migrated/Moved from the study area
- Lost to follow-up
- Sponsor study termination
- Other (specify)

*If a participant is withdrawn from the study because he/she/the participant's parent(s)/LAR(s) has withdrawn consent and the reason for withdrawal was provided, the investigator must document this reason in the eCRF.

Participants who are withdrawn from the study because of AEs/SAEs must be clearly distinguished from participants who are withdrawn for other reasons. The Investigator will follow participants who are withdrawn from the study due to an AE/SAE until the event is resolved (see Section 10.3.8.2).

7.3. Lost to follow-up

A participant will be considered 'lost to follow-up' if he/she fails to return for scheduled visits and cannot be contacted by the study site. If a participant is unable to be reached, he/she will be considered to have withdrawn from the study.

Please refer to the SPM for a description of actions to be taken before considering the participant lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

Protocol waivers or exemptions are only permitted when necessary for the management of immediate safety concerns for the participant.

Immediate safety concerns should be discussed with the sponsor as soon as they occur or when the study team becomes aware of them. The purpose of this communication is to determine if the participant(s) should discontinue the study intervention.

Study procedures and their timing are summarised in the SoA (Section 1.3).

All screening evaluations must be completed, and the results reviewed before confirming that potential participants meet all eligibility criteria.

The investigator will maintain a log of all participants screened. All relevant information, such as confirmation of eligibility and reasons for screening failure will be mentioned in this screening log.

Procedures conducted as part of routine clinical management (e.g. haematologic profiles), and obtained before the participant/participant's parent(s)/LAR(s) signed the ICF, may be used for screening and/or for establishing a clinical baseline (provided the procedure met protocol specified criteria and was performed within the time frame defined in the SoA) (Section 1.3).

The SPM provides the investigator and site personnel with detailed administrative and technical information that does not impact participant safety.

If local regulations allow and quality of study procedures is maintained, participant(s) can be offered remote visits (e.g., telemedicine, home visits) for the collection of biological samples and/or safety data/safety assessment(s)/study intervention administration. These remote visits must be performed by qualified study staff. Refer to the Schedule of Activities for the timing of these visits. Details of how these visits will be conducted are outlined in the SPM.

The following procedures can be performed remotely/virtually (refer to the Glossary of terms for the definitions of telemedicine, remote and virtual visits):

- Safety follow-up may be performed by telemedicine which will use secure video conferences, phone calls, and a web portal and/or mobile application as a way of communicating with the participant and monitoring the participant's progress. In addition, qualified study staff may also identify AEs and report them to the investigator for evaluation.
- Biological samples may be collected remotely by qualified study staff. Biological samples should be collected only if they can be processed in a timely manner and appropriately stored until the intended use.
- Administration of study intervention can be performed remotely/at participant's home by qualified study staff if appropriate storage conditions for the study intervention can be ensured. Furthermore, appropriate medical treatment must be readily available during 60 minutes after dosing in case of anaphylaxis, syncope.
- If despite best efforts, it is not possible to perform the Visits within the interval predefined in Table 5 of the protocol (e.g., due to COVID pandemic restrictions or potential delays in releasing outcomes of safety reviews), then the interval may be extended for the specified Visits up to a maximum length of days as presented in Table 13.

Table 13 Intervals between study visits during special circumstances

Interval	Planned visit interval	Allowed interval range*
Stage 1 – Adults 18 to 50 years of age – Groups 1 and 2		
Visit 1→Visit 4	28 days	28 days-38 days
Stage 1 – Adults 18 to 50 years of age – Group 1		
Visit 4→Visit 5	56 days	50 days-64 days
Visit 5→Visit 7	28 days	28 days-38 days
Stage 1 – Adults 18 to 50 years of age – Group 2		
Visit 4→Visit 5	140 days	130 days-150 days
Visit 5→Visit 7	28 days	28 days-38 days
Stage 2 – Adults 18 to 50 years of age		
Visit 1→Visit 3	28 days	28 days-38 days
Visit 3→Visit 4	56 days	50 days-70 days
Visit 4→Visit 6	28 days	28 days-38 days
Stage 2 – Children 24 to 59 months of age		
Visit 1→Visit 3	28 days	28 days-38 days
Visit 3→Visit 4	56 days	50 days-70 days
Visit 4→Visit 6	28 days	28 days-38 days
Stage 2 – Infants 9 months of age		
Visit 1→Visit 3	28 days	28 days-38 days for dose-finding cohort
Visit 3→Visit 4	56 days	50 days-70 days
Visit 4→Visit 6	28 days	28 days-38 days for dose-finding cohort
Visit 6→Visit 7	140 days	130 days-150 days
Visit 7→Visit 9	28 days	28 days-38 days

*In case a participant is out of window for a particular visit, the next Visit should be scheduled based on the originally planned protocol visit intervals and allowed interval ranges

8.1. Immunogenicity assessments

Biological samples will be used for research planned in the protocol and for purposes related to the improvement, development and quality assurance of the laboratory tests described in this protocol.

Findings in this or future studies may make it desirable to use samples acquired in this study for research not planned in this protocol. In this case, all participants in countries where this is allowed will be asked to give consent to allow GSK or a contracted partner, to use the samples for further research. The further research will be subject to prior institutional review board/independent ethics committee (IRB/IEC) approval, if required by local legislation.

Information on further research and its rationale can be obtained from GSK.

Sample testing will be done in accordance with the recorded consent of the individual participant/participant's parent(s)/LAR(s).

By default, collected samples will be stored for a maximum of 20 years. This storage period begins when the last participant performs the last study visit. This timeline can be adapted based on local laws, regulations or guidelines requiring different timeframes or procedures. In all cases, the storage period should be aligned with participant's consent. These additional requirements must be formally communicated to, discussed and agreed with GSK.

8.1.1. Biological samples

Table 14 summarises the collection of blood in the study.

The total amount of blood scheduled to be collected during the study is as follows:

- Adults 18 to 50 years of age in Stage 1: up to 265 mL
- Adults 18 to 50 years of age in Stage 2: ~76 mL
- Children 24 to 59 months of age in Stage 2: ~74 mL
- Infants 9 months of age in Stage 2: ~51 mL

In case of abnormal safety laboratory results, repeat or unscheduled samples may be taken.

Up to 150 mL of blood will be collected 28 days after the second study intervention administration in Stage 1 from 20 participants who gave specific informed consent for the purpose of extraction of peripheral blood mononuclear cells (PBMC) and sera, and considered eligible by the site staff (refer to the SPM for further details). Extracted materials will be used to generate monoclonal antibodies, which may play a role in protection against *Shigella* infection. This blood collection is not linked to any endpoint in this study but related to separate exploratory research on monoclonal antibodies against *Shigella*.

Table 14 Biological samples

Sample type	Quantity	Unit	Timepoint	Participants sampled
Blood for serum preparation	Approximately 10 mL/Visit for antibody determination; 10 mL/Visit for assay development; 15 mL for safety screening, 10 mL for pre/post-vaccination safety assays	mL	As scheduled; refer to Table 1 and Figure 1	All adults in Stage 1
	Approximately 10 mL/Visit for antibody determination, 12 mL for safety screening, 6 mL for pre/post-vaccination safety assays		As scheduled; refer to Table 2 and Figure 1	All adults in Stage 2
	Approximately 10 mL/Visit for antibody determination, 10 mL for safety screening, 6 mL/Visit for pre/post-vaccination safety assays		As scheduled; refer to Table 3 and Figure 1	All children in Stage 2
	Approximately 5 mL/Visit for antibody, 9 mL for safety screening, 2 mL/Visit for pre/post-vaccination safety assays		As scheduled; refer to Table 4 and Figure 1	All infants in Stage 2
Blood for PBMC extraction and serum preparation (exploratory research purposes)	Up to 150 mL		As scheduled; refer to Table 1 and Figure 1	Up to 20 participants in Stage 1; conditional on participant's specific consent

PBMC=peripheral blood mononuclear cell

8.1.2. Laboratory assays

Table 15 Laboratory assays

Assay type	System	Component	Method	Laboratory*
Humoral Immunity (Antibody determination)	Serum			
		Anti-*S. sonnei* LPS/OAg total IgG	GVGH ELISA (Stage 1 secondary objective and Stage 2 primary and secondary objectives)	GVGH or GVGH designated laboratory
		Anti-*S. flexneri* 1b LPS/OAg total IgG		
		Anti-*S. flexneri* 2a LPS/OAg total IgG		
		Anti-*S. flexneri* 3a LPS/OAg total IgG		
		Bactericidal antibodies against *S. sonnei*	SBA	
		Bactericidal antibodies against *S. flexneri* 1b		
		Bactericidal antibodies against *S. flexneri* 2a		
		Bactericidal antibodies against *S. flexneri* 3a		
		Anti-*S. sonnei* LPS total IgG	Reference ELISA (Stage ***2*** tertiary objective)	WRAIR
		Anti-*S. flexneri* 1b LPS total IgG		
		Anti-*S. flexneri* 2a LPS total IgG		
		Anti-*S. flexneri* 3a LPS total IgG		
		Measles	ELISA (Stage 2 secondary objective)	GVGH designated laboratory
		Rubella		

ELISA=enzyme-linked immunosorbent assay; GVGH=GSK Vaccines Institute for Global Health; LPS=lipopolysaccharide; IgG=Immunoglobulin G; OAg=O-Antigen; SBA=serum bactericidal assay; WRAIR=Walter Reed Army Institute of Research
*Refer to the list of clinical laboratories for details.

Exploratory systems serology analysis assays, panel SBA, opsonophagocytosis assays, and anti-*Shigella* conserved protein IgG ELISA assays will be performed by the GVGH laboratory or a laboratory designated by GVGH.

Please refer to the Section 10.2 for a brief description of the assays performed in the study.

The addresses of clinical laboratories used for sample analysis are provided in a separate document accompanying this Study Protocol.

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

8.1.3. Immunological read-outs

Table 16 Immunological read-outs

Blood sampling timepoint		Subset name (refer to Section 6.3.5)	No. participants	Component
Type of contact and timepoint	**Sampling timepoint**			
Adults in Europe (Stage 1) Group 1				
Visit 1 (Day 1), Visit 5 (Day 85)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 113)	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Adults in Europe (Stage 1) Group 2				
Visit 1 (Day 1), Visit 5 (Day 169)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 197	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
Adults in Africa (Stage 2)				
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	N/A	20	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-Vaccination	Stage 2 Adults	15	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri 1b*
				Bactericidal antibodies against *S. flexneri 2a*
				Bactericidal antibodies against *S. flexneri 3a*
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	N/A	20	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 1b* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 2a* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 3a* LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	Stage 2 Adults	15	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri 1b*
				Bactericidal antibodies against *S. flexneri 2a*
				Bactericidal antibodies against *S. flexneri 3a*
Children in Africa (Stage 2)				
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	NA	40	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-Vaccination	Stage 2 Children	25	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	NA	40	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	Stage 2 Children	25	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
Infants in Africa (Stage 2)-Safety cohort				
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	60	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
Visit 1 (Day 1)			***60***	***Anti-S. sonnei LPS total IgG (WRAIR)***
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-vaccination	Stage 2 Infant Safety Cohort	40	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	Post-vaccination	N/A	60	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)			***60***	***Anti-S. sonnei LPS total IgG (WRAIR)***
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	Post-vaccination	Stage 2 Infant Safety Cohort	40	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Infants in Africa (Stage 2)-Dose-finding cohort				
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	328	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 1 (Day 1)				Measles IgG
				Rubella IgG
Visit 3 (Day 29), Visit 6 (Day 113), Visit 9 (Day 281)	Post-vaccination			Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 9 (Day 281)				Measles IgG
				Rubella IgG

GVGH=GSK Institute for Global Health; IgG=immunoglobulin G; LPS=lipopolysaccharide; N/A=not applicable; OAg=O-Antigen; WRAIR=Walter Reed Army Institute of Research

8.1.4. Cytology

Not applicable

8.1.5. Clinical safety laboratory assessments

Refer to the Section 10.2.1 for the list of clinical laboratory safety assessments required by the protocol. These assessments must be conducted according to the SPM and the SoA (Section 1.3).

8.1.6. Immunological correlates of protection

No generally accepted immunological correlate of protection (CoP) has been demonstrated so far for the antigens used in the candidate vaccine. For *S. sonnei*, the GVGH ELISA level equivalent threshold values of 1:800 and 1:1600 to Cohen thresholds are not externally validated [Cohen, 2022], and have been chosen for the purpose of comparison to previous GVGH studies.

For the measles antigen, the conventional CoP is measles IgG antibody titre seroprotection threshold of ≥200 mIU/mL as measured by ELISA at 28 days after the second MR-VAC vaccination. For the rubella antigen, the conventional CoP is rubella IgG antibody titre seroprotection threshold of ≥4 IU/mL as measured by ELISA at 28 days after the second MR-VAC vaccination.

The immunological assay results will be communicated to the investigator not later than 12 months after the visit date at which sampling allows the assessment of whether the threshold for protection has been reached.

No other antigen measurements that can assess an immunological CoP are planned in this study.

Refer to the Section 6.6 for details regarding treatment for non-responders.

8.2. Safety assessments

The investigator and his/her designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE. This includes the assessment of COVID-19 cases as per the WHO definition [WHO, 2020]. The investigator and designees are responsible for following up AEs that are serious, considered related to the study intervention or the study, or that caused the participant's withdrawal from the study interventions or the study.

8.2.1. Pre-vaccination procedures

8.2.1.1. Collection of demographic data

Record demographic data such as date of birth (month and year only for adults 18 to 50 years of age; day, month, and year for children 18 to 59 months of age and infants 9 months of age), sex, and race in the participant's eCRF. Collection of sex and race data is necessary to assess and monitor the diversity of the trial participants, and to determine if the trial participants are truly representative of the impacted population. Date of birth is collected to stratify the population and determine the impact of the study intervention by age. For infants, the date of birth is also used to ensure that participants are eligible for inclusion in the study.

8.2.1.2. Medical/vaccination history

All participants:

Obtain the participant's medical history by interviewing the participant/parent(s)/LAR(s) and/or review of the participant's medical records. Record any pre-existing conditions, signs and/or symptoms present prior to the first dose of study intervention in the eCRF.

As part of the participant/parent(s)/LAR(s) interview during Screening and to evaluate whether the participant meets exclusion criterion of known exposure to *Shigella*, the investigator or delegate should assess whether there are any medical records showing confirmed microbiological diagnosis of *Shigella* diarrhoea in the lifetime of the participant. Additionally, any medical history suggestive of *Shigella* diarrhoea should be obtained based on the following features and the medical judgement of the investigator:

- History of watery diarrheoa with or without blood associated with crampy abdominal pain
- History of fever, toxicity, and anorexia
- Features suggestive of Haemorrhagic Uraemic Syndrome
- Features of acute abdomen following diarrhoeal illness
- History of similar illness in other household members

Adults 18 to 50 years of age in Europe:

As part of the participant/LAR(s) interview during Screening and to evaluate whether the participant meets exclusion criterion of known exposure to *Shigella*, the investigator or delegate should obtain participant travel history (within 2 years) to countries highly endemic for *Shigella* (refer to Section 10.9 for the list of countries) or occupational exposure (within 3 years) to *Shigella*. Examples of occupational exposure could include caring for non-human primates, working in child day-care facilities, and male commercial sex workers who have sex with men.

Infants 9 months of age and children 24 to 59 months of age only:

Obtain the participant's vaccination history by interviewing the parent(s)/LAR(s) and/or review of the participant's medical records.

8.2.1.3. Physical examination

A complete physical examination will be performed at Screening and on each vaccination visit before the vaccination (refer to Section 1.3). The physical examination will be performed by the investigator and clinically relevant findings should be recorded in the participant source documents and in the eCRF. Physical examination includes assessment of body temperature (preferably axillary) and resting vital signs (systolic/diastolic blood pressure, pulse rate [heart rate for children and infants], and respiratory rate). On days of vaccination, body temperature and vital signs will be measured before and 60 minutes after vaccination. If the investigator determines that the participant's health on the days of vaccination temporarily precludes vaccination, the visit will be rescheduled as possible by the study site.

A symptom-directed physical examination of the participant will be performed on visits where no vaccination is administered (refer to Section 1.3). The nature of the physical examination will be driven by any specific symptoms reported by the participant. The symptom-directed physical examination will not be recorded in the eCRF. However, any abnormalities detected that meet the definition of AE (refer to Section 10.3) will be recorded as such in the eCRF.

For Stage 1 and Stage 2 adults, height and weight will be recorded only at screening and entered in the source documents and in the eCRF. For Stage 2 children and infants, height (length for infants) and weight will be recorded at all study visits and entered in the source documents. Only height (length for infants) and weight values recorded at screening will be entered in the eCRF.

Treatment of any abnormalities observed during any physical examinations must be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

8.2.1.4. Pregnancy test

Female participants of childbearing potential must perform a urine pregnancy test before the administration of any dose of study intervention. Pregnancy testing must be done even if the participant is menstruating at the time of the study visit. The study intervention may only be administered if the pregnancy test is negative.

In case a pregnancy is suspected by the investigator, a pregnancy test may be performed at other Visits.

Refer to the Section 10.4.3.1 for the information on study continuation for participants who become pregnant during the study.

8.2.1.5. Warnings and precautions to vaccination

Warnings and precautions to administration of study intervention must be checked at each visit with planned administration of study intervention.

Refer to the IB for the altSonflex1-2-3 vaccine. Refer to the approved product label/package for all other vaccines.

8.2.2. Study holding rules and safety monitoring

Holding rules (refer to Table 17) are introduced to ensure a well-controlled exposure to the investigational candidate vaccine and to prevent participants from being exposed to any unnecessary safety risks in this FTIH study. The holding rules will point attention to safety signals and indicate requirements for formal committee reviews. The committee will decide whether additional participants can be exposed to the vaccine. If the investigator becomes aware of a holding rule being met, he/she will suspend vaccination and will inform GSK immediately.

Safety during the study will be monitored on an ongoing basis by the Safety Review Team (SRT), a cross-functional internal GSK committee, that will monitor safety data in a blinded manner. In addition, a separate internal Safety Review Committee (iSRC) will be established to monitor not only the safety throughout the study but also to specifically recommend continuation of dose-escalation procedures based on the safety results. The iSRC, which will be comprised of study-independent GSK members and an external expert (non-GSK employee), will review unblinded data. *Ad hoc* safety evaluations by the iSRC may be called at any time if there are any suspected safety concerns. The organisation of the iSRC and the specific roles of its members will be outlined in the iSRC Charter.

If a safety concern is identified during safety monitoring that may change the benefit-risk profile of 1 or more dose levels, appropriate actions will be taken. This may include termination of administration of 1 or more dose levels and enrollment with approximately 82 participants per group only for the remaining dose level(s) that would continue to be evaluated, in the dose-finding cohort and/or the corresponding dose-escalation steps**.**

8.2.2.1. Staggered vaccination

The investigator must await the receipt of favourable written documentation of the iSRC or SRT safety evaluations (approval to start vaccinations) after every safety evaluation before starting further dosing of study participants. Screening procedures may continue, to facilitate the enrolment of remaining participants.

Participants will always be vaccinated sequentially and closely observed for at least 60 minutes after vaccination.

Table 17 describes all planned safety evaluation. Blinded reviews (safety evaluations #1, #4, #6, and #7) will be jointly blinded reviews of all available safety data performed by both the iSRC Chair and the SRT. During these reviews, if no holding rules or safety concerns are identified, review of unblinded data by iSRC will not be required and the

study can proceed. If there are any safety concerns, the iSRC will convene and perform unblinded review of relevant data, proceeding as specified in the iSRC Charter. Unblinded safety evaluations (#2, #3, #5, #8, and #9) will be reviews of all the available safety data performed by the iSRC. The iSRC will also determine whether any of the predefined study holding rules are met.

Table 17 Safety evaluation schedule

Safety evaluation	Safety data reviewed*	Vaccination approval to
Stage 1: Adults 18 to 50 years of age		
#1: Blinded**	Up to 7 days after 1st vaccination of first 12 participants	Administration of 2nd vaccination to first 12 participants + administration of 1st vaccination to subsequent 90 participants in Stage 1
#2: Unblinded	Up to 28 days after 1st vaccination of all 102 participants	Administration of 1st vaccination to adults in Stage 2
Stage 2: Adults 18 to 50 years of age		
#3: Unblinded	Up to 7 days after 1st vaccination in all adults in Stage 2	Administration of 1st vaccination with Dose B to children
Stage 2: Children 24 to 59 months of age		
#4: Blinded**	Up to 7 days after 1st vaccination in all children with Dose B	Administration of 1st vaccination with Dose C to children, Administration of 2nd vaccination with Dose B to children
#5: Unblinded	Up to 28 days after 1st vaccination in all children with Dose C	Administration of 1st vaccination with Dose A to infants
Stage 2: Infants 9 months of age		
#6: Blinded**	Up to 7 days after 1st vaccination in all infants with dose A in the safety cohort	Administration of 1st vaccination with Dose B to infants in the safety cohort, Administration of subsequent vaccinations with Dose A to infants in the safety cohort
#7: Blinded**	Up to 7 days after 1st vaccination in all infants with Dose B in the safety cohort	Administration of 1st vaccination with Dose C to infants in the safety cohort, Administration of subsequent vaccinations with Dose B to infants in the safety cohort
#8: Unblinded	Up to 28 days after 1st vaccination in all infants with Dose C in the safety cohort	Administration of 1st vaccination to infants in the dose-finding cohort, Administration of subsequent vaccinations to infants of Dose C in the safety cohort
#9: Unblinded	Up to 7 days after the 1st vaccination in the dose-finding cohort of all infants who were vaccinated in the first 7 weeks since the 1st vaccination in the dose-finding cohort	Administration of subsequent vaccinations to infants in the dose-finding cohort

*Safety data will be cumulatively reviewed at each safety evaluation

**During blinded safety evaluations, if there are any concerns that would require the evaluation of unblinded safety data, an unblinded iSRC safety evaluation will occur that will decide on the succeeding administration of the vaccine or to begin the next step of the trial

8.2.2.1.1. Stage 1 (Europe)

One hundred and two adults 18 to 50 years of age will receive 2 vaccinations with either Dose C of altSonflex-2-3 or altSonflex Placebo using a 3-month interval schedule or 2 vaccinations with Dose C of altSonflex1-2-3 or altSonflex Placebo using a 6-month interval schedule (2:2:1:1 randomisation ratio). The first 12 adults will receive a first vaccination with either altSonflex1-2-3 or altSonflex Placebo using a staggered enrolment approach, with a time interval of at least 60 minutes between participants. When this is complete, all available safety data from the 7 days after the first vaccination will be reviewed in a blinded manner to decide if the study can continue with the second vaccination in the first 12 participants and all vaccinations for the remaining 90 participants (Table 17, safety evaluation #1). The subsequent 90 adults will be vaccinated in parallel. When all adult participants in Stage 1 have received the first vaccinations, all available safety data from 28 days after the first vaccination will be reviewed in an unblinded manner to decide if the study can continue to Stage 2 in Africa (Table 17, safety evaluation #2).

8.2.2.1.2. Stage 2 (Africa)

Adults 18 to 50 years of age

After approval, 20 adults will receive 2 vaccinations of either Dose C (dose previously administered to adults in Europe in Stage 1) of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). The control vaccine will be MENVEO for the first vaccination and BOOSTRIX for the second vaccination. The first 5 adults will receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 adults will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter.***

When all adults in Stage 2 have received the first vaccination, all available safety data from 7 days after vaccination will be reviewed in an unblinded manner to decide if the study can continue with vaccination of the children safety cohort (Table 17, safety evaluation #3).

Children 24 to 59 months of age

After approval, 20 children will receive 2 vaccinations of either Dose B of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). The control vaccine will be MENVEO for the first vaccination and TYPHIM VI for the second vaccination. The first 5 children will receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 children will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

When all children have received the first vaccination with Dose B, all available safety data from 7 days after first vaccination will be reviewed in a blinded manner to decide if the study can continue with administration of the second vaccination in children with dose B or the control. At the same time, the safety evaluation will decide whether vaccination with Dose C can also be initiated in children (Table 17, safety evaluation #4).

After approval, 20 children will receive 2 vaccinations of either Dose C of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). The control vaccines will be MENVEO for the first dose and TYPHIM VI for the second dose. The first 5 children will then receive the first vaccination in a staggered manner with a time interval of at least 60 minutes between participants. The subsequent 15 children will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

When all children have received the first vaccination with Dose C, all available safety data from 28 days after first vaccination will be reviewed in an unblinded manner to decide if the study can continue with administration of the first vaccination with Dose A to infants of the safety cohort (Table 17, safety evaluation #5).

Infants 9 months of age

After approval, 20 infants will receive 3 primary vaccinations of either Dose A of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ***INFANRIX HEXA*** for the third vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter.***

When all infants have received the first vaccination with Dose A, all available blinded safety data from 7 days after vaccination will be reviewed to decide if the study can continue with Dose B or control and succeeding vaccinations with Dose A or control (Table 17, safety evaluation #6).

After approval, 20 infants will receive 3 primary vaccinations with either Dose B or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ***INFANRIX HEXA*** for the third vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

When all infants have received the first vaccination with Dose B, all available safety data from 7 days after vaccination will be reviewed in a blinded manner to decide if the study can continue with Dose C or control and subsequent vaccinations with Dose B or control (Table 17, safety evaluation #7).

After approval, 20 infants will receive 3 primary vaccinations with either Dose C or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ***INFANRIX HEXA*** for the third vaccination. The first 5 infants will then receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

When all infants have received the first vaccination with Dose C, safety data from 28 days after vaccination will be reviewed in an unblinded manner to decide if the study can continue to the dose-finding cohort and subsequent vaccinations with Dose C or control (Table 17, safety evaluation #8).

After approval, a total of 328 infants will be vaccinated with either Dose A, Dose B, or Dose C of the altSonflex1-2-3 candidate vaccine, or with a control vaccine (1:1:1:1 randomisation ratio). The vaccination schedule will be the same as in the infant safety cohort. However, in this cohort, the EPI vaccination with MR-VAC will be given concomitantly with the first vaccination and with the third vaccination.

Seven weeks after the first infant in the dose-finding cohort received the first vaccination, all available safety data (i.e., data collected up to the 7 days after the first vaccination of infants in the dose-finding cohort and all available safety data from the safety cohort of adults and children) will be reviewed in an unblinded manner to decide if the study can continue with the second vaccination of infants in the dose-finding cohort (Table 17, safety evaluation #9). This approach is to avoid having infants who receive the first vaccination in the dose-finding cohort being out of window for the second vaccination, in consideration for recruitment and operational timelines.

8.2.2.2. Outcome of safety evaluation

- If no safety signal/concern is observed, the favourable outcome of the safety evaluations will be documented and provided in writing, authorising the investigator to start vaccination of participants with the subsequent dose as well as enrolment and vaccination of the remaining participants of the next step of the study.
- In case the investigator becomes aware of one or more holding rules (refer to Section 8.2.2.3), he/she should immediately inform GSK of the event (refer to Section 8.3.3.1 for contact information).
- If a safety signal is observed during blinded or unblinded safety data reviews or if any of the holding rules 2a-b (refer to Table 18) is met, the iSRC Chair (or his/her representative) is responsible for the urgent communication to the GVGH PP, including any rationale for a decision to put the vaccination on hold or not.
- The GVGH PP will be accountable for notifying all investigators of the decision whether to suspend, modify or continue the conduct of the study on all groups or on selected groups.

8.2.2.3. Study holding rules

The safety holding rules are defined in Table 18. Holding rules 1a-d will be assessed by the investigator on a continuous basis irrespective of the number of participants enrolled and meeting any of these holding rules will trigger a hold of vaccination irrespective of number of participants enrolled and/or timing of the event relative to vaccination. Holding rules 2a-b will be assessed by the iSRC during the safety evaluations on unblinded data. The evaluation for the percentage of participants needed to trigger a hold will be based on the number of participants receiving altSonflex1-2-3 at the time of the safety evaluation.

These holding rules have been written under the assumption that the safety data from all participants will be available. If the data from some of the participants are not available (i.e., in case a participant is lost to follow-up), then the holding rules will be assessed on a pro-rata basis.

Of note, no formal holding rules will be applied for other safety data such as missed visits due to vaccine-related AEs, Grade 1 and Grade 2 solicited and unsolicited AEs in the 7-day follow-up period, and unsolicited AEs collected from Day 8 after vaccination. However, these data, if available, will also be reviewed by the SRT and iSRC in order to allow for an overall assessment of the benefit/risk ratio of vaccination.

Table 18 Study holding rules

Holding rule	Event	Number or percentage of participants needed to trigger a hold	
		Safety cohorts	Dose-finding cohorts
1a	Death or any life-threatening SAE regardless of causality	≥1	
1b	Any non-life-threatening SAE that can be reasonably attributed to the vaccination	≥1	
1c	Any withdrawal from the study (by investigator or participant request) following a Grade 3 AE that can be reasonably attributed to the vaccination	≥1	
1d	Fever >40°C that can be reasonably attributed to the vaccination OR Any administration-site or systemic solicited event leading to hospitalisation OR Necrosis at the injection site, each within the 7-day (days 1-7) post-vaccination period	≥1	
2a	Any Grade 3 solicited administration-site event (lasting 48h or more) in an investigational group*, within the 7-days (Days 1-7) post-vaccination period	10%**	10%**
2b	Any Grade 3 unsolicited AE in an investigational group*, that can be reasonably attributed to the vaccination, within the 7-day (day 1-7) post-vaccination period or Any Grade 3 abnormality in prespecified haematological or biochemical laboratory parameters*** in an investigational group* within the 7-day (day 1-7) post-vaccination period	≥1 in all safety cohorts, except for ≥3 for Dose 2 data evaluation in Stage 1**	10%**

AE=adverse event; SAE=serious adverse event
*Investigational group refers to an individual study group receiving the altSonflex1-2-3 vaccine
In-stream ***and planned* blinded monitoring will evaluate the total number of participants vaccinated at the time of the safety evaluation (candidate vaccine and control recipients) and will trigger an *ad hoc* iSRC if the percentage threshold is reached. The iSRC will confirm if the holding rule has been met based on the number of participants who have received altSonflex1-2-3 vaccine in the specific investigational group.
***Please refer to Section 10.8 for the toxicity grading scales of laboratory parameters. Laboratory parameters that are not included in the toxicity grading scales will not be graded.

If the investigator becomes aware of a holding rule being met, he/she must suspend administration of the study intervention and inform GSK immediately (e.g., holding rules 1a-d). Refer to the Table 24 for contact information.

GSK will inform the investigator if holding rules 2a or 2b are met.

The following communication sequence must be followed:

- The concerned site staff must put vaccination on hold.
- The concerned site staff must immediately inform their study contact defined in Table 24.
- All informed site staff of other site will send back an email to the GVGH Study Delivery Lead (SDL) and GVGH PP to acknowledge receipt of the information.
- The GSK-GVGH clinical team in collaboration with the GSK GS Department will further evaluate the case with the iSRC and GSK Global Safety Board and will take the decision to stop or to restart the vaccination. All site staff will be informed about that final decision by the GVGH SDL and GVGH PP.

8.3. Adverse Events (AEs), Serious Adverse Events (SAEs), and other safety reporting

8.3.1. Time period and frequency for collecting AE, SAE, and other safety information

Table 19 Timeframes for collecting and reporting of safety information in adults in Stage 1

Event	SCR-D-*28* to D-1*	VAC 1 D1	D8	D29	VAC 2 D85/D169	D92/D176	D113/D197
Administration-site and systemic solicited events		D1 to D7			D85/D169 to D91/D175		
Unsolicited AEs		D1 to D28			D85/D169 to D112/D196		
AE/SAEs leading to withdrawal from the study		D1 to D113/D197					
SAEs		D1 to D113/D197					
SAEs related to the study intervention		D1 to D113/D197					
SAEs related to study participation or concurrent GSK medication/vaccine	D-28 to D113/D197						
Pregnancies			D1 to D113/D197				
Any other event of interest in the study			D1 to D113/D197				

AE: adverse event; D: Day; SAE=serious adverse event; SCR: screening; VAC: vaccination;
* i.e., consent obtained.

Table 20 Timeframes for collecting and reporting of safety information in adults in Stage 2

Event	SCR-D-21 to D-1*	VAC 1 D1	D8	D29	VAC 2 D85	D92	D113
Administration-site and systemic solicited events		D1 to D7			D85 to D91		
Unsolicited AEs		D1 to D28			D85 to D112		
AEs/SAEs leading to withdrawal from the study		D1 to D113					
SAEs		D1 to D113					
SAEs related to the study interventions		D1 to D113					
SAEs related to study participation or concurrent GSK medication/vaccine	D-21 to D113						
Pregnancies		D1 to D113					
Any other event of interest in the study		D1 to D113					

AE: adverse event; D: Day; SAE=serious adverse event; SCR: screening; VAC: vaccination;
* i.e., consent obtained.

Table 21 Timeframes for collecting and reporting of safety information in Children in Stage 2

Event	SCR-D-21 to D-1*	VAC 1 D1	D8	D29	VAC 2 D85	D92	D113
Administration-site and systemic solicited events		D1 to D7			D85 to D91		
Unsolicited AEs		D1 to D28			D85 to D112		
AEs/SAEs leading to withdrawal from the study		D1 to D113					
SAEs		D1 to D113					
SAEs related to the study interventions		D1 to D113					
SAEs related to study participation or concurrent GSK medication/vaccine	D-21 to D113						
Any other event of interest in the study		D1 to D113					

AE: adverse event; D: Day; SAE=serious adverse event; SCR: screening; VAC: vaccination;
* i.e., consent obtained.

Table 22 Timeframes for collecting and reporting of safety information in Infants in Stage 2

Event	SCR* D-21 to D-1*	VAC 1 D1 D8 D29	VAC 2 D85 D92 D113	VAC 3 D253 D260 D281
Administration-site and systemic solicited events		D1 to D7	D85 to D91	D253 to D259
Unsolicited AEs		D1 to D28	D85 to D112	D253 to D280
AEs/SAEs leading to withdrawal from the study		D1 to D281		
SAEs		D1 to D281		
SAEs related to the study interventions		D1 to D281		
SAEs related to study participation or concurrent GSK medication/vaccine	D-21 to D281			
Any other event of interest in the study		D1 to D281		

AE: adverse event; D: Day; SAE=serious adverse event; SCR: screening; VAC: vaccination;
* consent obtained.

The investigator or designee will record and immediately report all SAEs in enrolled participants to the sponsor or designee via the Expedited AE Reporting Form. Reporting should, under no circumstances, occur later than 24 hours after the investigator becomes aware of an SAE, as indicated in Section 10.3.10. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available. SAEs in non-enrolled participants will be reported as spontaneous SAEs as per local routine pharmacovigilance.

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting periods defined in Table 19. Investigators are not obligated to actively seek AEs or SAEs from former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention, the investigator will promptly notify the study contact for reporting SAEs mentioned in the Table 24.

8.3.2. Method of detecting AEs and SAEs, pregnancies, and other events

Detection and recording of AE/SAE/pregnancies are detailed in Section 10.3.8.

Assessment of AE/SAE intensity, causality and outcome are described in Section 10.3.9.

Open-ended and non-leading verbal questioning of participants/participants' parent(s)/LAR(s) is the preferred method of acquiring information related to an AE/SAE/ pregnancy.

8.3.2.1. Clinically significant abnormal laboratory findings

The investigator must review the laboratory report, document that he/she did so, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. Clinically significant abnormal laboratory findings are those which are not associated with an underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

- All laboratory tests with clinically significant abnormal values reported during the study or within 28 days after the last dose of study intervention should be repeated until the values return to normal/baseline, or until they are no longer considered significantly abnormal by the investigator. Refer to the Section 10.3.6 for more information on clinically abnormal laboratory assessments that qualify as an AE or SAE.
- In case abnormal values are not considered clinically significant by the investigator, additional evaluations could be requested by the GVGH PP.
- If abnormal values do not return to normal/baseline after an interval judged reasonable by the investigator, the aetiology of the abnormal value should be identified, and the sponsor notified.

8.3.3. Regulatory reporting requirements for SAEs, pregnancies, and other events

Once an investigator (or designee) becomes aware that a study participant has experienced an SAE/pregnancy, it must be reported to GSK using the required documentation and within the timeframes mentioned in Table 23. This is essential for meeting GSK legal obligations and ethical responsibilities for participant safety and the safety of a study intervention under clinical investigation.

For SAEs, the investigator must always provide an assessment of causality at the time of the initial report, as defined in the Section 10.3.9.2.

Local regulatory requirements and sponsor policy for preparation of an investigator safety report of SUSAR must be followed. These reports will be forwarded to investigators, as necessary.

The sponsor has the legal responsibility to notify local authorities/regulatory agencies about the safety of an investigational study intervention. The sponsor will comply with country-specific regulatory requirements related to safety reporting to the regulatory authority, IRB/IEC, and investigators.

Please refer to the Section 10.3.10 for further details regarding the reporting of SAEs/ pregnancies.

Table 23 Timeframes for submitting SAE and pregnancy reports to GSK

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	24 hours*	electronic pregnancy report	24 hours*	electronic pregnancy report

SAE=serious adverse event
*Timeframe allowed after receipt or awareness of the information by the investigator/site staff.
‡The investigator will be required to confirm 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.

8.3.3.1. Contact information for reporting SAEs, pregnancies, and study holding rules

Table 24 Contact information for reporting SAEs, pregnancies, and study holding rules

Study contact for questions regarding SAEs and pregnancies Refer to the local study contact information document	**Study contact for reporting of study holding rules** As soon as the investigator is aware that a holding rule is met, he/she must immediately inform the GVGH SDL and the GVGH PP.
Back up study contact for reporting SAEs and pregnancies 24/24 hour and 7/7-day availability: **GSK Clinical Safety & Pharmacovigilance** Belgium and Kenya sites: Email address: ogm28723@gsk.com Fax: +32 2 656 80 09 t	**Back up study contact for escalation of holding rules** If the GVGH SDL or GVGH PP cannot be reached, the investigator should contact the GVGH Head of Clinical Development & Regulatory Affairs

GVGH PP: GSK Vaccines Institute for Global Health Project Physician; GVGH SDL: GSK Vaccines Institute for Global Health Study Delivery Lead; SAE: serious adverse event

8.3.4. Treatment of adverse events

Any medication administered for the treatment of an SAE should be recorded in the Expedited Adverse Event Report of the participant's eCRF screen (refer to Section 10.3.10.1).

8.3.5. Participant card

The investigator (or designee) must provide the participant/participant's parent(s)/LAR(s) with a "participant card" containing information about the clinical study. The participant/participant's parent(s)/LAR(s) must be instructed to always keep the participant card in his/her/their possession for the duration of the study. In an emergency, this card serves to inform the responsible attending physician/LAR/care giver/family member that the participant is in a clinical study and that relevant information may be obtained by contacting the investigator or his/her back up.

8.3.6. Medical device deficiencies

Some of the comparator control vaccines used in this study are combination products constituted of a biologic product in a medical device (pre-filled syringes). Refer to the Glossary of terms for the definition of combination product and medical device deficiency.

8.3.6.1. Detection, follow-up, and prompt reporting of medical device deficiency

The investigator is responsible for the detection, documentation and prompt reporting of any medical device deficiency occurring during the study to GSK. This applies to any medical device provided for the conduct of the study.

Device deficiencies will be reported to GSK within 24 hours after the investigator determines that the event meets the protocol definition of a device deficiency. Refer to Section 10.6 for definitions and details on recording and reporting of these events.

The investigator will ensure that follow-up includes any additional investigations to elucidate the nature and/or related of the device deficiency to the incident. Follow-up applies to all participants, including those who discontinue study intervention or the study.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator and reported to GSK within 24 hours.

8.3.6.2. Regulatory reporting of medical device deficiency when used as combination product

The investigator will promptly report all device deficiencies occurring with any medical device provided for use in the study to GSK. GSK has a legal responsibility to notify appropriate regulatory authorities and other entities about safety information linked to medical devices being used in clinical studies. Refer to section 10.6.3 for details of reporting.

The investigator, or responsible person according to local requirements (e.g., the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4. Pharmacokinetics

Not applicable

8.5. Genetics and/or Pharmacogenomics

To minimise any risk of autoimmune reactions, blood samples collected for safety during Screening will undergo sampling for the HLA-b27 genetic marker. Participants with a positive test result will not be included in the study. In case of participant rescreening, this test should not be repeated.

8.6. Biomarkers

Not applicable

8.7. Immunogenicity

Not applicable

8.8. Health outcomes

Not applicable

9. STATISTICAL CONSIDERATIONS

9.1. Statistical hypotheses

Statistical hypothesis testing will be applied to the following primary hypothesis:

- To identify the preferred dose of each component of the altSonflex1-2-3 (low, medium, or high) for infants 9 months of age in Africa (Stage 2).

The statistical tests will be chosen according to the dose-response relationship that will be observed for each of the 4 components of the vaccine. See Section 9.2.2 for more details.

No statistical hypothesis testing will be performed for all other objectives.

9.2. Sample size determination

Approximately 550 participants will be randomised to achieve 486 evaluable participants. Participants who withdraw from the study will not be replaced.

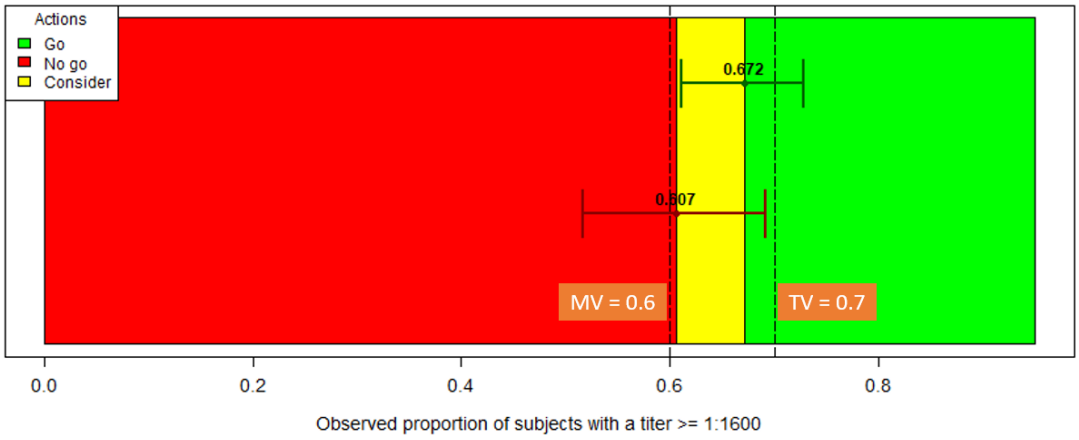
9.2.1. Stage 1 Quantitative Decision Making

A QDM approach will be used to support the Go/No-Go decision to proceed to Stage 2. The endpoint measuring number of adults in Europe 18 to 50 years of age in Stage 1 achieving a GVGH ELISA level equivalent to 1:1600 titre against *S. sonnei* LPS at Day 29 was selected as the most clinically relevant for decision making. A Minimum Value (MV) of 60% and a Target Value (TV) of 70% for participants in the vaccine group with

GVGH ELISA level below 1:1600 titre against *S. sonnei* LPS at baseline (Day 1) achieving such a threshold at Day 29 have been defined for the clinical development programme. MV and TV are the critical values that are the basis for defining the threshold limits (vertical solid lines in Figure 2) that will determine the Go/No-Go decision in the context of the QDM approach.

Approximately 102 participants will be randomised to achieve 61 evaluable participants in the vaccine arm of Stage 1**.** Assuming a 10% drop-out rate, including removal of participants with GVGH ELISA level ≥1:1600 titres at baseline (Day 1), in Stage 1 and therefore 61 evaluable participants in the participants receiving altSonflex1-2-3, and defining a MV of 60% and TV of 70% of participants achieving an equivalent 1:1600 ELISA level against *S. sonnei* titres, an observed percentage of participants ≥67.2% achieving the equivalent 1:1600 titre threshold ensures with a more than 80% probability that the true percentage of participants is greater than or equal to 60% (MV). This leads to a Go decision (green zone in Figure 2). An observed percentage of participants ≤67.2% (yellow and red zones) will trigger a comprehensive analysis of all available immunogenicity data across serotypes and/or after the second vaccination in Stage 1 to make the Go/No-Go decision.

Figure 2 QDM Critical values and decisions



MV=minimum value; TV=target value

9.2.2. Stage 2 Dose-finding cohort

Approximately 328 participants will be randomised to achieve 288 evaluable participants.

The statistical tests to identify the preferred dose among the 3 different dose levels (Dose A [low], Dose B [medium], and Dose C [high]; see Figure 3) have been defined according to the potential dose-response shapes that will be observed for each of the 4 components. Immunogenic responses will be measured by ELISA as GMC and two-sided 95% Confidence Intervals (CI) of anti-serotype specific *Shigella* LPS/OAg serum IgG for all 4 serotypes (*S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a). The following selection strategy has been predefined for each of the foreseen scenarios of the dose-response shapes, the following:

1. Monotonic negative response
   - No tests needed, the preferred dose is Dose A.

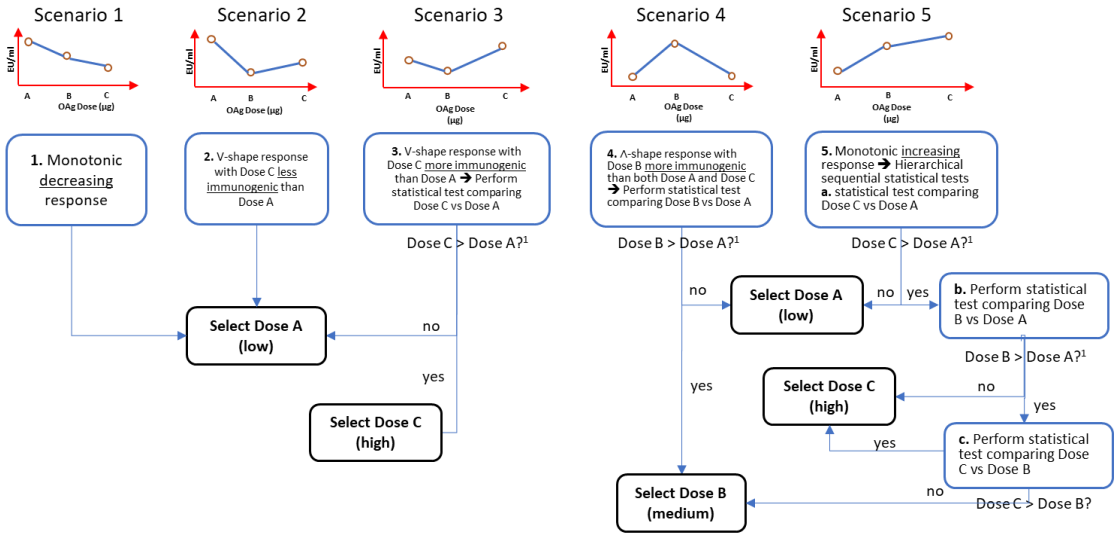
2. V-shape response with Dose C less immunogenic than Dose A:
   - No tests needed, the preferred dose is Dose A.
3. V-shape response with Dose C more immunogenic than Dose A
   - If the difference between Dose C and Dose A is statistically significant, the preferred dose is Dose C.
   - If the difference between Dose C and Dose A is not statistically significant, the preferred dose is Dose A.
4. ∧ shape response with Dose B more immunogenic than Dose A and C
   - If the difference between Dose B and Dose A is statistically significant, the preferred dose is Dose B.
   - If the difference between Dose B and Dose A is not statistically significant, the preferred dose is Dose A.
5. Monotonic positive response
   - Proceed with 3 following hierarchical sequential tests (fixed sequence procedure)
     a. If the difference between Dose C and Dose A is not statistically significant, the preferred dose is Dose A. If the difference between Dose C and Dose A is statistically significant, the difference between Dose B and Dose A will be tested (see point b. below).
     b. If the difference between Dose B and Dose A is not statistically significant, the preferred dose is Dose C. If the difference between Dose B and Dose A is statistically significant, the difference between Dose C and Dose B will be tested (see point c. below).
     c. If the difference between Dose C and Dose B is not statistically significant, the preferred dose is Dose B. If the difference between Dose C and Dose B is statistically significant, the preferred dose is Dose C.

In the absence of previous studies on the altSonflex1-2-3 formulation, the minimum increment to define the preferred dose has been chosen by the GVGH clinical team to be a 2-fold increase between groups 28 days after the third vaccination. The selection of the preferred dose will be conditional on an acceptable safety profile for that dose.

For the 3 groups of infants 9 months of age receiving the two-dose primary vaccination schedule at the 3 different doses in the dose-finding cohort, a sample size of 72 evaluable participants will have ~94% power to detect at least 2-fold ratio between GMTs at a 0.05 two-sided significance level using a two-independent sample t-test, and assuming a within-group standard deviation of log10 ELISA equal to 0.5. Assuming a ~12% drop-out rate in Stage 2, a total of approximately 82 infants will have to be enrolled in each of the 3 groups of the dose-finding cohorts.

The rationale for the selection strategy used to identify the preferred dose is that in the absence of an appreciable increment in response between different doses (above defined as 2-fold increase in ratio of GMC), the preferred formulation is the one with lowest amount of OAg. The sample size of 82 infants per group has been calculated according to scenarios 3, 4, and 5 where at least one t-test is performed to select the preferred dose. In case a dose level is discontinued due to safety concerns, at most 1 t-test comparing higher to lower dose will be performed to select the preferred dose. If scenarios 1 or 2 occur, no statistical test will be performed, and Dose A will be selected as the preferred one.

Figure 3 Dose-response scenarios and statistical tests for identifying the preferred dose of the altSonflex1-2-3 vaccine



[1]from t-test for independent samples assuming 2-fold difference on GMT and SD on log10 (EU) = 0.5
EU = ELISA unit; GMT = Geometric Mean Titer; OAg = O-Antigen

Table 25 shows the power achieved with the target sample size (72 evaluable participants per group) to demonstrate a 2-fold increase in ratio of GMC.

Table 25 Power calculation

Two-sample t-tests assuming equal variance							
Power	**n**	**k**	**N**	**Alpha**	**Beta**	**Difference**	**S**
0.9463	46	3	138	0.05	0.0537	0.301	0.40
0.9465	58	3	174	0.05	0.0535	0.301	0.45
0.9482	72	3	216	0.05	0.0518	0.301	0.50

n=number of participants for each group receiving vaccine; k=number of vaccinated groups; N=total number of participants for each group receiving vaccine; Alpha=2-sided alpha; Difference=smallest difference between any 2 means that is to be detectable by the experiment on a log10-scale (i.e., 0.301 on log10-scale equals to 2-fold on original scale); S=within-groups standard deviation on the log10-transformed values.

The values in Table 25 have been generated with PASS 2019 v19.0.1 Two-Sample T-Tests Assuming Equal Variance module. The below assumptions were used:

- α-level for two-sided t-test: 0.05.
- Power (1–β) for one t-test: 0.9457. This is equivalent to an 0.80 (0.9457^4 =0.80) power for 4 simultaneous independent t-test (one for each antigen).

- Minimum Detectable Difference between groups: 0.301 on a log10 scale, equivalent to a 2-fold difference in ratios of GMCs.
- Within-group Standard Deviation: 0.40, 0.45, 0.50, on a log10 scale (range of values based on previous studies with *S. sonnei* 1790GAHB vaccine).

9.3. Analysis sets

Table 26 Analysis sets

Analysis set	Description
Enrolled	All participants who entered the study (who were randomised or received study intervention or underwent a post-screening study procedure). Note: participants who never passed screening (screening failures) and participants screened but who never enrolled into the study (met eligibility criteria but were not needed) are excluded from the Enrolled analysis set.
Exposed	All participants who received at least 1 dose of the study intervention. The allocation in a group is based on the administered intervention.
Full Analysis	All participants who received at least 1 dose of the study intervention and have post-vaccination immunogenicity data. The allocation in a group is done in function of the randomised intervention. The Full Analysis Set for immunogenicity will be defined by time point.
Per Protocol	All eligible participants who received all doses as per protocol, had immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination. The Per Protocol Set (PPS) for immunogenicity will be defined by time point.
Unsolicited Safety	All participants who received at least 1 dose of the study intervention (Exposed Set) that report unsolicited AEs/report not having unsolicited AEs. The allocation in a group is based on the administered intervention.
Solicited Safety	All participants who received at least 1 dose of the study intervention (Exposed Set) who have solicited safety data.

9.3.1. Criteria for elimination from analysis

If the participant meets one of the criteria mentioned below or ones listed in the Section 7.1.1, he/she may be eliminated from per-protocol analysis.

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) used during the study period.
- Use of 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 (for adult subjects) or ≥ 0.5 mg/kg/day (for paediatric subjects), or equivalent. Inhaled and topical steroids are allowed.
- Us of any long-acting immune-modifying drugs administered at any time during the study period (e.g., infliximab).
- Use of any vaccine not foreseen by the Study Protocol administered during the entire period of participant's study participation (refer to Section 5.2.2).

- Use of foreseen vaccines outside the recommended time window (refer to Section 5.2.2).
- Use of any immunoglobulins and/or any blood products administered during the study period.
- Any drug and/or alcohol abuse.
- Occurrence of 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.
- Occurrence of a serious chronic or progressive disease according to judgement of the investigator (e.g., neoplasm, insulin dependent diabetes, cardiac, renal, or hepatic disease).
- Occurrence of any malignancy or lymphoproliferative disorder.
- Occurrence of any confirmed or suspected immunosuppressive or immunodeficient condition, based on physical examination.
- Unblinding after any study intervention has been administered.

Other criteria for elimination from analyses will be reported in the SAP.

9.4. Statistical analyses

The primary analysis will be based on the Per Protocol Set (PPS).

9.4.1. Primary endpoints

The analysis on primary objective will be based on the PPS for analysis of immunogenicity. If, in any study group and at any timepoint, the percentage of vaccinated participants with serological results excluded from the PPS for analysis of immunogenicity is 10% or more, a second analysis based on the Full Analysis Set will be performed to complement the per-protocol analysis.

Endpoint	Statistical analysis methods
Primary immunogenicity	**Within-groups assessment** For each study group (Dose A, Dose B, Dose C, and control vaccine) of the 9-month infant dose-finding cohort, the GM***C*** with 95% CI of anti-serotype specific *Shigella* LPS/OAg* total IgG as measured by GVGH ELISA will be calculated at 28 days after third vaccination. **Between groups assessment** For each of the 4 serotypes in 9-month infant dose-finding cohort, the preferred dose at 28 days after third vaccination will be identified according to the scenarios reported in Figure 3: • Scenario 1 and 2: Dose A • Scenario 3: t-test comparing Dose C with Dose A • Scenario 4: t-test comparing Dose B with Dose A • Scenario 5: hierarchical sequential with the 3 following tests: 1) t-test comparing Dose C with Dose A: if null hypothesis is rejected then 2) t-test comparing Dose B with Dose A: if null hypothesis is rejected then 3) t-test comparing Dose B with Dose C. In addition to scenarios reported in Figure 3, in case of a dose level discontinuation

Endpoint	Statistical analysis methods
	• if the lower dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then the lower dose will be selected as the preferred dose without performing any statistical test • if the higher dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then a t- test comparing the higher with lower dose will be performed to select the preferred dose Unadjusted Ratios of GMCs with 95% CI between groups for all pairwise comparisons will be calculated. *_S. sonnei_, _S. flexneri_ 1b, _S. flexneri_ 2a, and _S. flexneri_ 3a serotypes will be tested.
Primary safety	• The percentage of participants with at least 1 administration-site solicited event or unsolicited AE, with at least 1 systemic solicited event or unsolicited AE and with any AE during the 7-day or 28-day follow-up period will be tabulated by group after each vaccine dose and overall. • The percentage of doses associated with at least 1 administration-site solicited event or unsolicited AE, with at least 1 systemic event and unsolicited AE, and with any AE will be tabulated for the overall vaccination course. The same calculations will be performed for symptoms rated as Grade 3. • The percentage of participants reporting each individual solicited administration-site and systemic event (any Grade and Grade 3) during the 7-day solicited follow-up period will be tabulated. The percentage of doses associated with each individual solicited administration-site and systemic event will be tabulated for the overall vaccination course. • Occurrence of fever will be reported as number and percentages of participants reporting temperature ≥38°C in 0.5°C cumulative increments during the 7-day follow-up period. This will be tabulated for each group after each dose. • The percentage of participants with at least one reported unsolicited AE during the 28-day follow-up period after each vaccination as classified by the Medical Dictionary for Regulatory Activities (MedDRA) preferred term will be tabulated. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs according to relationship to vaccination. • Percentage of participants reporting SAEs and AEs/SAEs leading to withdrawal from the study during the participant period participation will be tabulated. The detailed listings will also be produced. • For each group and for haematological, renal, and hepatic panel test results at 7 days after each vaccination: – The percentage of participants having haematological, renal, and hepatic results below or above the laboratory normal ranges will be tabulated by time point. – The maximum Grade (refer to Section 10.8) from Day 1 up to the end of the participant's participation will be tabulated. The verbatim reports of unsolicited symptoms will be reviewed by a physician and the signs and symptoms will be coded according to the MedDRA Dictionary for AE Terminology.

Safety analysis will be based on the Exposed, Solicited Safety and Unsolicited Safety sets.

9.4.2. Secondary endpoints

Endpoint	Statistical analysis methods
Secondary immunogenicity	**Within-groups assessment** For each study group in participants in Europe, for each sampling timepoint, and for each antigen, the following statistics will be computed: • Unadjusted GMCs with 95% CI of anti-serotype specific *Shigella* LPS/OAg* total IgG as measured by GVGH ELISA • Within-participant geometric mean ratios (GMRs) will be computed for GMCs with 95% CI at each time point after vaccination versus baseline (Day 1) • Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by GVGH ELISA For each study group in participants in Africa, for each sampling timepoint, and for each antigen, the following statistics will be computed: • Unadjusted GMCs with 95% CI of anti-serotype specific *Shigella* LPS/OAg* total IgG as measured by GVGH ELISA • Within-participant GMRs will be computed for GMCs with 95% CI at each time point after vaccination versus baseline (Day 1) • Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by GVGH ELISA For each study group within 9-month dose-finding infant cohort, for the sampling timepoint before the first vaccination and 28 days after the second MR-VAC vaccination, the following statistics will be computed for the anti-measles and anti-rubella antigens, as measured by ELISA: • Unadjusted GMCs with 95% CI of anti-measles and anti-rubella antibodies • Percentage of participants with measles seroresponse of ≥150 mIU/mL and ≥200 mIU/mL, with exact 95% CI • Percentage of participants with rubella seroresponse of ≥4 mIU/mL and ≥10 mIU/mL, with exact 95% CI. For all the above, within-participant GMRs will be computed for GMT or GMC with 95% CI at each time point after vaccination versus baseline (Day 1). The distribution of antibody concentrations for each antigen will be displayed using reverse cumulative distribution curves. **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.

9.4.3. Tertiary endpoints

Endpoint	Statistical Analysis Methods
Tertiary immunogenicity	For participants in ***Africa in the safety cohorts***, for each sampling timepoint, and for each antigen the following statistics will be computed, as measured by reference ELISA: • Unadjusted GMTs with 95% CI of anti-serotype specific *Shigella* LPS * total IgG • Within-participant GMRs will be computed for GMTs with 95% CI at each time point after vaccination versus baseline (Day 1) • Number and percentage of participants with 1:800 and 1:1600 equivalent titres with exact 95% CI From the subset defined by 25% randomly chosen participants from the Stage 1 adults 18 to 50 years of age, "Stage 1 Adults Subset", the following statistics will be computed: • Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before, 15 days after first vaccination, and 28 days after each vaccination From the subset defined by randomly chosen participants from the Stage 2 adults 18 to 50 years of age ("Stage 2 Adults Subset"), the following statistics will be computed: • Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination From the subset defined by randomly chosen participants from the Stage 2 children 24 to 59 months of age ("Stage 2 Children Subset"), the following statistics will be computed: • Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination From the subset defined by randomly chosen participants from the Stage 2 infant 9 month of age safety cohort ("Stage 2 Infant Safety Cohort Subset"), the following statistics will be computed: • Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination For all participants in the 9-month infant dose-finding cohort, the following statistics will be computed • Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before and 28 days after each vaccination For all the above, within-participant GMRs will be computed for GMT with 95% CI at each time point after vaccination versus baseline (Day 1). Additional exploratory immunology including system serology analysis, panel SBA, opsonophagocytosis assays, and anti-*Shigella* conserved protein IgG ELISA assays will be performed on the aforementioned subsets of 25% randomly chosen Stage 1 adult participants and on the infant dose-finding cohort. Results will be reported by group and timepoint using descriptive summary statistics. This analysis will be described in a dedicated SAP. *S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.

9.5. Interim analyses

Tables and listings for the iSRC reviews will be prepared by an independent unblinded external statistician outside of the project with the support of an Independent Data Analysis Centre (IDAC) to maintain the study blind and will be documented in a statistical analysis report. Only the outcome of the iSRC review (yes/no safety concerns) will be communicated to the study team. Additional details on iSRC reviews are described in the iSRC Charter.

To accelerate evaluation of vaccine immunogenicity in the target population and facilitate planning of future studies, 4 study interim analyses and 1 final analysis (Stage 1 and Stage 2) will be performed as described in Section 9.5.1.

9.5.1. Sequence of analyses

All analyses before unblinding will be conducted on cumulative data as clean as possible.

The sequence of interim and final analyses will be the following:

a. Stage 1 interim analysis at Day 29 (28 days after first vaccination) on immunogenicity in all Stage 1 participants. This analysis will support a QDM approach, which will be part of a GO/NO-GO decision for the study to proceed to Stage 2; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

b. Stage 1 interim analysis at Day 197 on immunogenicity and safety data on all Stage 1 participants in order to obtain early immunogenicity and safety data from Stage 1; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

c. Stage 2 interim analysis at Day 113 on immunogenicity data from all infant safety cohorts (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 safety cohorts, in order to obtain early immunogenicity data analysis and facilitate planning for subsequent clinical development, to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

d. Stage 2 interim analysis at Day 113 on immunogenicity data from infant dose-finding cohort (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 cohorts, to facilitate planning for subsequent clinical development, to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

e. Final analysis including Stage 1 final analysis at Day 197 and Stage 2 final analysis at Day 281 on all immunogenicity and safety data once Stage 2 of the trial is concluded; to be carried out by the GVGH Project Statistician and GSK Statistical Analysts.

In case of a No-Go decision after the first (a) or second (b) interim analyses, a final analysis will be performed at Day 197 on immunogenicity and safety data of all Stage 1 participants, to be carried out by GVGH Project Statistician and GSK Statistical Analysts.

The Clinical Study Report (CSR) will contain at least the final analyses of all primary and secondary endpoints (in case of a Go decision). Only Stage 1 endpoints will be part of the CSR in case of a No-Go decision (interim analyses “a” and “b”).

Data for tertiary endpoints which are available at the time of planned analyses will be included in the interim analyses. Data for tertiary endpoints that become available at later timepoints will be documented in annex(es) to the final CSR or in an integrated final CSR.

9.5.2. Statistical consideration for interim analysis

No adjustment of Type I error will be performed since no statistical hypothesis testing will be conducted prior to unblinding.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, ethical, and study oversight considerations

10.1.1. Regulatory and ethical considerations

- This study will be conducted in accordance with the protocol and with:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organisations of Medical Sciences International Ethical Guidelines
  - Applicable International Council of Harmonisation (ICH) GCP Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator for review and approval. These documents will be signed and dated by the investigator before the study is initiated.
- Any protocol amendments will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
  - Notifying the IRB/IEC of SAE(s) or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2. Financial disclosure

Investigators and sub-investigators must provide the sponsor with sufficient, accurate financial information, as requested, to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

10.1.3. Informed consent process

The investigator or his/her representative must fully explain the nature of the study to the participant/participant's parent(s) or his/her LAR(s) and answer all questions regarding the study.

Participants/participants' parent(s)/LAR(s) must be informed that their participation is voluntary.

Freely given and written or witnessed/thumb printed informed consent must be obtained from each participant and/or each participant's parent(s)/LAR(s)/witness, as appropriate, prior to participation in the study.

The content of the ICF must meet the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study centre.

The medical record must include a statement that written or witnessed/thumb printed informed consent was obtained before the participant was enrolled in the study and the date the consent was obtained. The authorised person obtaining the informed consent must also sign the ICF.

Participants must be re-consented if a new version of the ICF(s) or an ICF addendum is released during their participation in the study.

A copy of the ICF(s) must be provided to the participants/participants' parent(s)/LAR(s).

For participants who are rescreened, participants/participants' parent(s)/LAR(s) are required to sign a new ICF, only if there are changes to the original ICF. If there are no changes to the original ICF, participants/participants' parent(s)/LAR(s) should confirm that they still agree to be part of the study. This information should be captured in the participant source document.

10.1.4. Data protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets transferred to the sponsor will contain only the identifier. Name and any other information which would identify the participant will not be transferred.

The participants/participants' parent(s)/LAR(s) must be informed that:

- His/her personal/their child's study-related data will be used by the sponsor in accordance with local data protection law.
- His/her medical records/their child's may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

- GSK will ensure protection of the personal data of the investigator and site staff which is collected within the framework of and for the purpose of the study, in accordance with the Data Privacy Notice that will be sent to the site staff.

The participants/participants' parent(s)/LAR(s) must be notified about their rights regarding the use of their personal data in accordance with the data privacy section of the ICF.

10.1.5. Committees structure

The iSRC will consist of GSK safety experts, at least 1 external (non-GSK employee) member with expertise in clinical trials in developing countries, and a study-independent statistician. The iSRC Charter includes members' roles and responsibilities.

The SRT is a cross-functional internal GSK committee responsible for ongoing safety monitoring of the entire project.

10.1.6. Dissemination of clinical study data

The key design elements of this protocol and results summaries will be posted on www.ClinicalTrials.gov, https://pactr.samrc.ac.za, and/or GSK Clinical Study Register in compliance with applicable regulations/GSK policy. GSK will aim to register protocols summaries prior to study start and target results summaries submission within 12 months of primary/ study completion date. Where external regulations require earlier disclosure, GSK will follow those timelines.

Where required by regulation, summaries will also be posted on applicable national or regional clinical trial registers.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report, and provided reasonable access to statistical tables, figures, and relevant reports. GSK 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 participants, as appropriate.

GSK will provide the investigator with the randomisation codes for their site only after completion of the full statistical analysis.

GSK intends to make anonymised patient-level data from this trial available to external researchers for scientific analyses or to conduct further research that can help advance medical science or improve patient care. This helps ensure the data provided by trial participants are used to maximum effect in the creation of knowledge and understanding.

10.1.7. Data quality assurance

The investigator should maintain a record of the location(s) of their respective essential documents, including source documents. The document storage system used during the trial and for archiving (irrespective of the type of media used) should provide for document identification, version history, search, and retrieval.

Essential trial documents may be added or removed where justified (in advance of trial initiation) based on their importance and relevance to the trial. When a copy is used to replace an original document (e.g., source documents, CRF), the copy should fulfil the requirements for certified copies (see Glossary of terms for the exact definition of essential documents and certified copies).

All participant data related to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The investigator must maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial participants (see Glossary of terms for the exact definition of source documents) that supports information entered in the eCRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source documents or certified copies for such review and inspection.

The sponsor or designee is responsible for the data management of this study including quality checking of the source data (see Glossary of terms for the exact definition of source data).

Study monitors will perform ongoing source data verification to confirm that data entered in the eCRF by authorised site personnel are attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data must be traceable, not obscure the original entry, and be fully explained if necessary (e.g., via an audit trail). The safety and rights of participants must be protected, and the study conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Quality tolerance limits (QTLs) will be predefined in the Risk Management Plan Study Risk Register (GVGH Study Risk-Based Quality Assessment Template) to identify systematic issues that can impact participant safety and/or the reliability of study results. These predefined parameters will be monitored during the study. Important deviations from the QTLs and remedial actions taken will be summarised in the CSR.

Trial records and source documents pertaining to the conduct of this study, including signed ICFs, must be retained by the investigator for 25 years from issuance of the final CSR/equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8. Source documents

Source documents provide evidence to establish the existence of the participant and substantiate the integrity of collected data. The investigator should maintain a record of the location(s) of their source documents.

Data transcribed into the eCRF from source documents must be consistent with those source documents; any discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definitions of what constitutes source data and documents can be found in the Glossary of terms.

10.1.9. Study and site start and closure

First act of recruitment

The first act of recruitment, defined by either the date of first contact with a potential study participant or date of publication of an advertisement for this specific study (whichever is earlier), will signify the official start of the study.

Study/Site termination

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at its sole discretion, provided there is sufficient notice given to account for all participants safe exit from study.

Regular closure of study sites will occur upon study completion. A study site is considered closed when all required data/documents and study supplies have been collected and a study site closure visit has been performed.

The investigator may initiate study site closure at any time, provided there is reasonable cause and enough notice in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines

- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development
- Total number of participants included earlier than expected

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organisation(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

At the end of the study, the investigator will:

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

10.1.10. Publication policy

GSK aims to submit the results of the study for publication in searchable, peer reviewed scientific literature within 18 months from the LSLV for interventional studies and follows the guidance from the International Committee of Medical Journal Editors.

10.2. Appendix 2: Clinical laboratory tests

Anti-*Shigella* serotype-specific antibody determination from serum:

Anti-*Shigella* serotype-specific IgG antibodies in serum will be measured by an ELISA assay developed by the GVGH laboratories (Siena, Italy), based on a method previously reported [Launay, 2017; Launay, 2019]. The assay will be performed in a laboratory designated by GVGH. The ELISA is performed using *S. sonnei*, *S. flexneri 1b*, *S. flexneri* 2a, or *S. flexneri* 3a LPS/OAg as coating antigen. For this assay, a standard curve is run in duplicate on each plate. Results are expressed in ELISA units (EU)/mL of serum. One unit equals the reciprocal of the dilution to give an optical density of 1.

Anti-*Shigella* serotype-specific IgG antibodies in serum will also be measured by ELISA in the WRAIR laboratories (only for sera from ***the safety cohorts in adults, children, and infants in Stage 2 up to 28 days after their third vaccination***), based on a method previously reported [Riddle, 2016]. The ELISA is performed using *S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, or *S. flexneri* 3a LPS as coating antigen. Sera are serially diluted, and results are reported as endpoint titres, defined as the reciprocal of the last dilution of serum giving an optical density value above the predefined assay cut-off.

Serum bactericidal activity in serum:

Serum bactericidal activity against *S. sonnei*, *S. flexneri* 1b*, S. flexneri* 2a, *S. flexneri* 3a, and other heterologous strains, will be measured by luminescence-based SBA, based on a method previously reported [Necchi, 2017; Rossi, 2020]. Results are expressed in serum titres, defined as serum dilutions giving 50% inhibition of bacterial growth (IC_{50}).

Opsonophagocytosis assay

Opsonophagocytic killing activity (OPK) against a panel of *Shigella* strains will be measured by a traditional colony factor unit-based method using HL60 or Thp-1 cells. Results are expressed in OPK titres, defined as serum dilutions giving 50% inhibition of bacterial growth (IC_{50}).

Anti-*Shigella* conserved protein IgG ELISA

An anti-*Shigella* conserved protein (e.g., invasion plasmid antigen) total IgG ELISA will be used to profile natural exposure to *Shigella*.

Measles and rubella ELISA:

Anti-measles and anti-rubella IgG antibodies will be determined by ELISA using commercial kits.

System serology assay panel:

A panel of assays will be used to conduct a system serology analysis, based on a previously reported method [Chung, 2015], to evaluate the following antibody properties and functional activities: antibodies subclassing, antibody affinity, antibody avidity, antibody dependent cellular phagocytosis, antibody dependent cellular cytotoxicity, antibody dependent NK cell activation, antibody dependent eosinophil degranulation, antibody dependent basophil degranulation, antibody dependent complement deposition, antibody dependent neutrophil activation/phagocytosis, antibody dependent dendritic cell phagocytosis, antibody dependent mucin binding, Fc receptor array, and antibody glycosylation.

10.2.1. Protocol required safety laboratory assessments

Table 27 Protocol required safety laboratory assessments

Population	Laboratory assessments	Parameters
Safety screening laboratory assays		
All participants	**Haematology**	• Leukocytes (white blood cells [WBC]) • Erythrocytes (red blood cells [RBC]) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Total bilirubin • Aspartic Aminotransferase (AST) • Alanine Aminotransferase (ALT) • γ-Glutamyl Transferase (γ-GT) • Lactic Dehydrogenase (LDH) • Alkaline Phosphatase (AP) • Total proteins • Glucose (random glucose) • Blood Urea • Creatinine • Sodium • Potassium
	Other screening tests	• Hepatitis B surface antigen • Hepatitis C virus antibodies • HIV antibodies (adults and children) or HIV DNA by PCR (infants only) • HLA-B27 • Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for women of childbearing potential) • Prothrombin time
Pre- and post-vaccination Visit (non-screening) safety laboratory assays		
Adults in Europe (Stage 1)	**Haematology**	• Leukocytes (WBC) • Erythrocytes (RBC) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Creatinine • AST • ALT

Population	Laboratory assessments	Parameters
	Other screening tests	• Urine hCG pregnancy test
Adults in Africa (Stage 2)	**Haematology**	• Leukocytes (WBC) • Erythrocytes (RBC) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Creatinine • AST • ALT
	Other screening tests	• Urine hCG pregnancy test
Children in Africa (Stage 2)	**Haematology**	• Leukocytes (WBC) • Erythrocytes (RBC) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Creatinine • AST • ALT
Infants in Africa (Stage 2)	**Haematology**	• Leukocytes (WBC) • Erythrocytes (RBC) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Creatinine • ***Blood urea*** • ***Sodium*** • ***Potassium*** • AST • ALT

ALT=alanine aminotransferase; AST=aspartate aminotransferase; DNA=deoxyribonucleic acid; hCG=human chorionic gonadotropin; IEC=independent ethics committee; IRB=institutional review board; PCR=polymerase chain reaction; RBC=red blood cell; SAE=serious adverse event; ULN=upper limit of normal; WBC=white blood cell
NOTES:
All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE.
Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

The tests detailed in Table 27 will be performed by the local site laboratories.

10.3. Appendix 3: Adverse Events: definitions and procedures for recording, evaluating, follow-up, and reporting Definition of AE

10.3.1. Definition of an AE

An AE is any untoward medical occurrence (an unfavourable/unintended sign – including an abnormal laboratory finding), symptom, or disease (new or exacerbated) in a clinical study participant that is temporally associated with the study intervention. The AE may or may not be considered related to the study intervention.

10.3.1.1. Events Meeting the AE Definition

- Significant or unexpected worsening or exacerbation of the condition/indication under study.
- 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 administration of the study intervention even though they may have been present before study start.
- Signs, symptoms, or the clinical sequelae of a suspected drug, disease, or other interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either the study intervention or a concurrent medication.
- Signs or symptoms temporally associated with administration of the study intervention.
- Signs, symptoms that require medical attention (e.g., hospital stays, physician visits and emergency room visits).
- Significant failure of an expected pharmacologic or biological action.
- Pre- or post- intervention events that occur as a result of protocol-mandated procedures (i.e., invasive procedures, modification of participant's previous therapeutic regimen).
- 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.
- AEs to be recorded as solicited AEs are described in the Section 10.3.3. All other AEs will be recorded as UNSOLICITED AEs.

10.3.1.2. Events NOT Meeting the AE Definition
• 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 participant before the first dose of study intervention. These events will be recorded in the medical history section of the eCRF. • Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed before signing the informed consent) that did not worsen from baseline. • Any clinically significant abnormal laboratory findings or other abnormal safety assessments associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

10.3.2. Definition of an SAE

An 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 participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.
c.	Requires hospitalisation or prolongation of existing hospitalisation Note: In general, hospitalisation signifies that the participant 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 out-patient setting. Complications that occur during hospitalisation are also considered AEs. The event will also be considered serious if a complication prolongs hospitalisation or fulfils any other serious criteria. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.
d.	Results in disability/incapacity 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, diarrhoea, 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 participant.
f.	Abnormal pregnancy outcomes (e.g., spontaneous abortion, foetal death, stillbirth, congenital anomalies, ectopic pregnancy).

g. Other situations

Medical or scientific judgement must be exercised in deciding whether reporting is appropriate in other situations. Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or require medical or surgical intervention to prevent one of the other outcomes listed in the above definition should 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; and convulsions that do not result in hospitalisation.

10.3.3. Solicited events

a. Solicited administration-site events

The following administration-site events will be solicited:

Table 28 Solicited administration-site events

All age groups
Pain
Redness
Swelling

b. Solicited systemic events

The following systemic events will be solicited:

Table 29 Solicited systemic events

Infant/Child (<6 years)	Adult
Fever	Fever

Note: participants/participants' parent(s)/LAR(s) will be instructed to measure and record the axillary temperature in the evening. If additional temperature measurements are taken at other times of the day, participants/participants' parent(s)/LAR(s) will be instructed to record the highest daily temperature in the diary card.

10.3.4. Unsolicited adverse events

An unsolicited adverse event is an adverse event that was not included in a list of solicited events using a Participant Diary. Unsolicited events must have been spontaneously communicated by a participant/participant's parent(s)/LAR(s) who has signed the informed consent. Unsolicited AEs include both serious and non-serious AEs.

Potential unsolicited AEs may be medically attended (i.e., symptoms or illnesses requiring a hospitalisation, or an emergency room visit, or visit to/by a health care provider). The participants/participant's parent(s)/LAR(s) will be instructed to contact the site as soon as possible to report medically attended event(s), as well as any events that, though not medically attended, are of participant/parental/LAR's concern. Detailed information about reported unsolicited AEs will be collected by qualified site personnel and documented in the participant's records.

Unsolicited AEs that are not medically attended or perceived as a concern by the participant/participant's parent(s)/LAR(s) will be collected during an interview with the participants/participant's parent(s)/LAR(s) and by review of available medical records at the next visit.

10.3.5. Adverse events of special interest

Not applicable.

10.3.6. Clinical laboratory parameters and other abnormal assessments qualifying as AEs or SAEs

In the absence of a diagnosis, abnormal laboratory findings assessments (e.g. clinical chemistry, haematology, urinalysis) or other abnormal results the investigator considers clinically significant will be recorded as an AE or SAE, if they meet the definition of an AE or SAE (refer to the Sections 10.3.1 and 10.3.2).

The investigator must exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding, or other abnormal assessment is clinically significant.

10.3.7. Events or outcomes not qualifying as AEs or SAEs

10.3.7.1. Pregnancy

Female participants who become pregnant after the first study intervention dose must not receive subsequent doses of the study intervention but may continue other study procedures at the discretion of the investigator.

While pregnancy itself is not considered an AE or SAE, any abnormal pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an SAE. Please refer to the Section 10.3.2 for definition of SAE.

10.3.8. Recording and follow-up of AEs, SAEs, and pregnancies

The participants/participants' parent(s)/LAR(s) will be instructed to contact the investigator immediately should they experience any signs or symptoms they perceive as serious.

When an AE/SAE occurs, it is the investigator's responsibility to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) related to the event. This includes the assessment of COVID-19 cases as per the WHO definition [WHO, 2020]. The investigator will then record all relevant information regarding an AE/SAE on the eCRF. The investigator may not send photocopies of the participant's medical records to GSK instead of appropriately completing the eCRF.

There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers will be blinded on copies of the medical records prior to submission to GSK.

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 instead of individual signs/symptoms.

A pDiary, hereafter referred to as Participant Diary, will be used in this study to capture solicited administration-site or systemic events. The participant should be trained on how and when to complete the Participant Diary.

Anyone who measures administration-site or systemic events and who will record the event in the Participant Diary should be trained on using the Diary. This training must be documented in the participant's source record. If any individual other than the participant/participant's parent(s)/LAR(s) is making entries in the Participant Diary, their identity must be documented in the Participant Diary/source records.

The investigator or delegate will transcribe the required information into the eCRF in English.

10.3.8.1. Time period for collecting and recording AEs, SAEs, and pregnancies

All AEs that occur during 28 days following administration of each dose of study intervention (Adults in Europe: Day 1 to Day 28 and Day 85/Day 169 to Day 112/Day 196; Adults in Africa: Day 1 to Day 28 and Day 85 to Day 112; Children in Africa: Day 1 to Day 28 and Day 85 to Day 112; Infants in Africa: Day 1 to Day 28, Day 85 to Day 112, and Day 253 to Day 280) must be recorded into the appropriate section of the eCRF, irrespective of their intensity or whether or not they are considered related to the study intervention.

10.3.8.2. Follow-up of AEs, SAEs, pregnancies, or any other events of interest

After the initial AE/SAE/pregnancy or any other event of interest, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilised, otherwise explained, or the participant is lost to follow-up.

Other non-serious AEs must be followed until resolution or until the participant is lost to follow-up.

10.3.8.2.1. Follow-up during the study

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

If a participant dies during their participation in the study or during a recognised follow-up period, GSK will be provided with any available post-mortem findings, including histopathology.

10.3.8.2.2. Follow-up after the participant is discharged from the study

The investigator will provide any new or updated relevant information to GSK on a previously reported SAE using an electronic Expedited Adverse Events Report and/or pregnancy report as applicable. The investigator is obliged to perform or arrange for the conduct of supplemental clinical examinations/tests and/or evaluations to elucidate the nature and/or causality of the SAE as fully as possible.

10.3.8.2.3. Follow-up of pregnancies

Pregnant participants 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 using the electronic pregnancy report and the Expedited Adverse Events Report if applicable. Generally, the follow-up period does not need to be longer than 6 to 8 weeks after the estimated date of delivery.

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

Furthermore, if the investigator becomes aware of any SAE occurring as a result of a post-study pregnancy AND it is considered by the investigator to be reasonably related to the study intervention, he/she must report this information to GSK as described in the Section 10.3.10.

10.3.8.3. Updating of SAE and pregnancy information after removal of write access to the participant's eCRF

When additional SAE or pregnancy information is received after write access to the participant's eCRF is removed, 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 Section 8.3.3.1) or to the GSK GS department within the defined reporting time frames specified in the Table 23.

10.3.9. Assessment of intensity and toxicity

10.3.9.1. Assessment of intensity

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

Table 30 Intensity scales for solicited events in infants and children less than 6 years of age

Infant/ Child (<6 years)		
Event	**Intensity grade**	**Parameter**
Pain at administration site	0	None
	1	Mild: Minor reaction to touch
	2	Moderate: Cries/protests on touch
	3	Severe: Cries when limb is moved/spontaneously painful
Redness at administration site		Greatest surface diameter in mm
Swelling at administration site		Greatest surface diameter in mm
Temperature*		Temperature in °C (with 1 decimal)

*Fever is defined as temperature ≥ 38.0°C. The preferred location for measuring temperature will be the axilla for all participants.

Table 31 Intensity scales for solicited events in adults

Adults		
Event	**Intensity grade**	**Parameter**
Pain at administration site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal everyday activities
	2	Moderate: Painful when limb is moved and interferes with everyday activities
	3	Severe: Significant pain at rest. Prevents normal everyday activities
Redness at administration site		Greatest surface diameter in mm
Swelling at administration site		Greatest surface diameter in mm
Temperature*		Temperature in °C (with 1 decimal)

*Fever is defined as temperature ≥ 38.0°C. The preferred location for measuring temperature will be the axilla for all participants.

In infants and children, the maximum intensity of local injection-site erythema/swelling will be scored as follows:

Redness/Swelling (Infants/Children)			
Grade 0 **Absent**	**Grade 1** **Mild**	**Grade 2** **Moderate**	**Grade 3** **Severe**
<1 mm	≥1 – ≤25 mm	>25 – ≤50 mm	>50 mm

In adults, the maximum intensity of local injection-site erythema/swelling will be scored as follows:

Redness/Swelling (Adults)			
Grade 0 Absent	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**
<20 mm	≥20 – ≤50 mm	>50 – ≤100 mm	>100 mm

In all participants, the maximum intensity of fever will be scored as follows:

Fever			
Grade 0 Absent	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**
<38.0°C	38.0°C – <39.0°C	≥39.0°C – ≤40.0°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 judgement.

The intensity should be assigned to one of the following categories:

1 (mild)	=	An AE which is easily tolerated by the participant, causing minimal discomfort, and not interfering with everyday activities.
2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities (In a young child, such an AE would, for example, prevent attendance at a day-care centre and would cause the parent(s)/LAR(s) to seek medical advice. In adults, such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.)

An AE that is assessed as Grade 3 (severe) should not be confused with an SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets 1 of the predefined outcomes as described in the Section 10.3.2.

10.3.9.2. Assessment of causality

The investigator must assess the relationship between study intervention and the occurrence of fever (solicited systemic event) and each unsolicited AE/SAE using clinical judgement. Where several different interventions were administered, the investigator should specify, when possible, if the fever or unsolicited AE/SAE could be causally related to a specific intervention. When a causal relationship to a specific study intervention cannot be determined, the investigator should indicate the fever or unsolicited AE/SAE to be related to all interventions.

Alternative possible causes, such as the natural history of underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to the study intervention will be considered and investigated. The investigator will also consult the IB and/or Summary of Product Characteristics and/or Prescribing Information for marketed products to assist in making his/her assessment.

Causality should be assessed by the investigator using the following question:

Is there a reasonable possibility that the fever or unsolicited AE may have been caused by the study intervention?

YES	:	There is a reasonable possibility that the study intervention contributed to the AE.
NO	:	There is no reasonable possibility that the AE is causally related to the administration of the study intervention. There are other, more likely causes and administration of the study intervention is not suspected to have contributed to the AE.

If an event meets the criteria to be determined 'serious' (see Section 10.3.2), additional examinations/tests will be performed by the investigator 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 study intervention, if applicable.
- An error in study intervention administration.
- Other cause (specify).

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report to GSK. However, it is very important to record an assessment of causality for every event before submitting the Expedited Adverse Events Report to GSK.

The causality assessment is 1 of the criteria used when determining regulatory reporting requirements. The investigator may change his/her opinion of causality after receiving additional information and update the SAE information accordingly.

10.3.9.3. Medically attended visits

For each solicited and unsolicited AE the participant experiences, the participant/ participant's parent(s)/LAR(s) will be asked if he/she/the participant received medical attention (defined as hospitalisation, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits). This information will be recorded in the in the paper diary/source documents and in the eCRF/Expedited Adverse Events Report as applicable.

10.3.9.4. 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).

10.3.10. Reporting of SAEs, pregnancies, and other events

10.3.10.1. Events requiring expedited reporting to GSK

Once an investigator becomes aware that an SAE has occurred in an enrolled participant, the investigator (or designee) must complete information in the electronic Expedited Adverse Events Report WITHIN 24 HOURS, even if the investigator does not have complete information on the SAE. It must be completed as thoroughly as possible, with all available details of the event.

The SAE report must be updated WITHIN 24 HOURS of the receipt of updated information on the SAE. The investigator will always provide an assessment of causality at the time of the initial report.

Refer to the Table 23 for the details on timeframes for reporting of SAEs and pregnancies.

The investigator will be required to confirm the review of SAE causality by ticking the 'reviewed' box in the electronic Expedited Adverse Events Report within 72 hours of submission of the SAE.

Refer to the Section 10.3.10.2 for information on backup systems in case the electronic reporting system does not work.

10.3.10.2. Backup system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designee) must fax or email a completed, dated and signed paper Expedited Adverse Events Report to the study contact for reporting SAEs (refer to the Sponsor Information) or to the GSK GS department within 24 hours of becoming aware of the SAE.

Investigator (or designee) must complete the electronic Expedited Adverse Events Report within 24 hours after the electronic reporting system is working again. The information reported through the electronic SAE reporting system will be considered valid for regulatory reporting purposes.

10.4. Appendix 4: Contraceptive guidance and collection of pregnancy information

10.4.1. Definitions

10.4.1.1. Woman of childbearing potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

10.4.1.1.1. Women not considered as women of childbearing potential

- Premenarchal

  Menarche is the first onset of menses in a young female. Menarche is normally preceded by several changes associated with puberty including breast development and pubic hair growth.

  Additional evaluation should be considered if a participant's fertility status is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention.

- Premenopausal female with ONE of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
  - Current bilateral tubal ligation or occlusion

Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

- Postmenopausal female

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

- Females on HRT and whose menopausal status is in doubt will be required to use a non-hormonal, highly effective contraception method if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrolment.

10.4.2. Contraception guidance

- Female participants of childbearing potential are eligible to participate if they agree to use a highly effective contraceptive method consistently and correctly according to the methods listed in GSK's list of highly effective contraceptive methods (Table 32).

Table 32 Highly effective contraceptive methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly*
Combined (oestrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation • Oral • Intravaginal • Transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation • Injectable • Oral
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
Vasectomised partner *(A vasectomised partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP, and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.)*
Male partner sterilisation prior to the female participant's entry into the study, and this male is the sole partner for that participant, *(The information on the male sterility can come from the site personnel's review of the participant's medical records; medical examination and/or semen analysis, or medical history interview provided by her or her partner.)*
Sexual abstinence (*Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)*

10.4.3. Collection of pregnancy information

10.4.3.1. Female participants who become pregnant

Refer to the Sections 8.3.1, 8.3.2, 10.3.8.1 and 10.3.8.3 for further information on detection, recording, reporting and follow-up of pregnancies.

Any female participant who becomes pregnant during the study will discontinue study intervention or be withdrawn from the study**.**

10.5. Appendix 5: Genetics

Genetic testing for HLA-b27 will be performed at Screening and only participants with a negative result will be allowed to participate in the study. The assay will be performed by the local site laboratory or a laboratory designated by the site. In case of participant rescreening, this test should not be repeated since the result is not expected to change in the participant lifetime.

10.6. Appendix 6: Definition of medical device AE, adverse device effect (ADE), serious adverse device effect (SADE) and unanticipated SADE (USADE)

10.6.1. Definition of medical device AE and ADE

- Medical device AE is any untoward medical occurrence, in a clinical study participant, users, or other persons, temporally associated with the use of study intervention whether considered related to a medical device or not. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medical device. This definition includes events related to the medical device or comparator and events related to the procedures involved.
- An ADE is an AE related to the use of a medical device. This definition includes any AE resulting from:
  - insufficient or inadequate instructions for use (i.e., user error), or
  - any malfunction of a medical device, or
  - intentional misuse of the medical device.

10.6.2. Definition of medical device SAE, SADE and USADE

A medical device SAE is any serious adverse event that:
a. Led to death
b. Led to serious deterioration in the health of the participant, that either resulted in: – A life-threatening illness or injury. The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe. – A permanent impairment of a body structure or a body function. – Inpatient or prolonged hospitalisation. Planned hospitalisation for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE. – Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function.
c. Led to foetal distress, foetal death or a congenital abnormality or birth defect
d. Is a suspected transmission of any infectious agent via a medicinal product
Serious Adverse Device Effect (SADE) definition
• A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event. • Any device deficiency that might have led to an SAE if appropriate action had not been taken or circumstances had been less fortunate.
Unanticipated SADE (USADE) definition
• An USADE (also identified as UADE in US Regulations 21 CFR 813.3), is a serious adverse device effect that by its nature, incidence, severity, or outcome has not been identified in the current version of the IB.

10.6.3. Recording and reporting of medical device AE, ADEs, SADEs and USADE

- Any device deficiency must be reported to GSK within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- Email/Facsimile transmission of the paper 'Medical device or combination product with device deficiency/incident report form' is the preferred method to transmit this information to the sponsor.
- Contacts for reporting can be found in Section 8.3.3.1
- GSK will review all device deficiencies, determine and document in writing whether they could have led to an SAE. These device deficiencies will be reported to the regulatory authorities and IRBs/IECs as required by national regulations.

10.7. Appendix 7: Country-specific requirements

Not applicable

10.8. Appendix 8: Grading scales for haematology and biochemistry safety laboratory assessments

10.8.1. Stage 1 – Adults (Europe)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine (female) – mg/dL	>0.96 – 1.7	>1.7 – 2.0	>2.0 – 2.5	>2.5 or requires dialysis
Creatinine (male) – mg/dL	>1.17 – 1.7	>1.7 – 2.0	>2.0 – 2.5	>2.5 or requires dialysis
Liver Function Tests –ALT, AST increase by factor	≥1.1 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10 x ULN	>10 x ULN
Haemoglobin (female) – g/dL	11.0 – <11.8	9.5 – <11.0	8.0 – <9.5	<8.0
Haemoglobin (male) – g/dL	12.5 – <12.9	10.5 – <12.5	8.5 – <10.5	<8.5
Haemoglobin (female/male) change from baseline value – g/dL	Any decrease – 1.5	>1.5 – 2.0	>2.0 – 5.0	>5.0
WBC increase – cell/mm^3	>9300 – 15 000	>15 000 – 20 000	>20 000 – 25 000	>25 000
WBC decrease – cell/mm^3	2500 – <3650	1500 – <2500	1000 – <1500	<1000
Platelets decrease (female) – cell/mm^3	125 000 – <171 000	100 000 – <125 000	25 000 – <100 000	<25 000
Platelets decrease (male) – cell/mm^3	125 000 – <149 000	100 000 – <125 000	25 000 – <100 000	<25 000
Neutrophils decrease – cell/mm^3	1500 – <1573	1000 – <1500	500 – <1000	<500
Lymphocytes decrease –cell/mm^3	750 – <1133	500 – <750	250 – <500	<250
Eosinophils – cell/mm^3	>273 – 1500	>1500 – 5000	>5000	Hypereosinophilic

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Food and Drug Administration (FDA) Guidance for Industry- Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [U.S. Department of Health and Human Services, 2007] and adjusted considering local normal ranges provided by the site. Parameters not included in the FDA grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

10.8.2. Stage 2 – Adults (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine	≥1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN OR Increase to 1.3 to <1.5 x participant's baseline	>1.8 – <3.5 x ULN OR Increase to 1.5 to <2.0 x participant's baseline	≥3.5 x ULN OR Increase of ≥2.0 x participant's baseline
Liver function tests – ALT, AST	≥1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Haemoglobin (female) – g/dL	9.5	8.5 – <9.5	6.5 – <8.5	<6.5
Haemoglobin (male) – g/dL	10.0 – <13.3	9.0 – <10.0	7.0 – <9.0	<7.0
WBC decrease (female) – cell/mm^3	2000 – <3000	1500 – <2000	1000 – <1500	<1000
WBC decrease (male) – cell/mm^3	2000 – <2700	1500 – <2000	1000 – <1500	<1000
Platelets decrease (female) – cell/mm^3	100 000 – <124 000	50 000 – <100 000	25 000 – <50 000	<25 000
Platelets decrease (male) – cell/mm^3	100 000 – <115 000	50 000 – <100 000	25 000 – <50 000	<25 000
Neutrophils decrease – cell/mm^3 (female)	800 – <962	600 – <800	400 – <600	<400
Neutrophils decrease (male) – cell/mm^3	800 – <870	600 – <800	400 – <600	<400
Lymphocytes decrease (female) – cell/mm^3	600 – <1264	500 – <600	350 – <500	<350
Lymphocytes decrease (male) – cell/mm^3	600 – <1098	500 – <600	350 – <500	<350

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

10.8.3. Stage 2 – Children (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine	≥1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN OR Increase to 1.3 to < 1.5 x participant's baseline	>1.8 – <3.5 x ULN OR Increase to 1.5 to < 2.0 x participant's baseline	≥3.5 x ULN OR Increase of ≥2.0 x participant's baseline
Liver function tests – ALT, AST	≥1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Haemoglobin (female & male) – g/dL	9.5 – <10.2	8.5 – < 9.5	6.5 – <8.5	<6.5
WBC decrease (female) – cell/mm^3	2000 – <4860	1500 – <2000	1000 – <1500	<1000
WBC decrease (male) – cell/mm^3	2000 – <5140	1500 – <2000	1000 – <1500	<1000
Platelets decreased (female) – cell/mm^3	100 000 – <189 000	50 000 – <100 000	25 000 – <50 000	<25 000
Platelets decrease (male) – cell/mm^3	100 000 – <202 000	50 000 – <100 000	25 000 – <50 000	<25 000
Neutrophils decrease (female) – cell/mm^3	800 – <1600	600 – <800	400 – <600	<400
Neutrophils decrease (male) – cell/mm^3	800 – <1540	600 – <800	400 – <600	<400

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

10.8.4. Stage 2 – Infants (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine ***– µmol/L***	***≥40.7 – 48.1***	***>48.1 – 66.6***	***>66.6 – <129.5***	***≥129.5***
Liver function tests – ALT, AST	≥1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Haemoglobin (female) – g/dL	9.5 – <10.2	8.5 – <9.5	6.5 – <8.5	<6.5
Haemoglobin (male) – g/dL	9.5 – <10.1	8.5 – <9.5	6.5 – <8.5	<6.5
WBC decrease (female) – cell/mm^3	2000 – <6480	1500 – <2000	1000 – <1500	<1000
WBC decrease (male) – cell/mm^3	2000 – <5980	1500 – <2000	1000 – <1500	<1000
Platelets decreased (female) – cell/mm^3	100 000 – <214 000	50 000 – <100 000	25 000 – <50 000	<25 000
Platelets decrease (male) – cell/mm^3	100 000 – <206 000	50 000 – <100 000	25 000 – <50 000	<25 000
Neutrophils decrease (female) – cell/mm^3	800 – <1270	600 – <800	400 – <600	<400
Neutrophils decrease (male) – cell/mm^3	800 – <1190	600 – <800	400 – <600	<400

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

10.9. Appendix 9: List of countries considered highly endemic for *Shigella*

Africa

- Cameroon
- Central African Republic
- Chad
- Democratic Republic of Congo
- Eritrea
- Ethiopia
- Lesotho
- Madagascar
- Mali
- Namibia
- Niger
- South Sudan
- Togo

Asia

- Bangladesh
- India
- Indonesia
- Nepal
- Pakistan
- Tajikistan

Oceania

- Papua New Guinea

10.10. Appendix 10: Abbreviations and Glossary of terms

10.10.1. List of abbreviations

ADE:	adverse device effect
AE:	adverse event
ALT:	alanine aminotransferase
AST:	aspartate aminotransferase
CHIM:	Controlled Human Infection Model
CI:	confidence intervals
CoP:	correlate of protection
CSR:	Clinical Study Report
DAIDS:	Division of AIDS
DNA:	deoxyribonucleic acid
eCRF:	electronic Case Report Form
ELISA:	enzyme-linked immunosorbent assay
EoS:	End of Study
EPI:	Expanded Program on Immunisation
FDA:	Food and Drug Administration, United States of America
FTIH	first time in human
GCP:	Good Clinical Practice
GMC:	geometric mean concentration
GMMA:	Generalised Modules for Membrane Antigens
GMR:	geometric mean ratio
GMT:	geometric mean titre
GS:	Global Safety (GSK)
GSK:	GlaxoSmithKline
GVGH:	GSK Vaccines Institute for Global Health
hCG:	Human chorionic gonadotropin
HIV:	human immunodeficiency virus
HLA:	human leukocyte antigen
HRT:	hormone replacement therapy
IB:	Investigator Brochure
ICF:	Informed Consent Form
ICH:	International Council on Harmonisation
IDAC:	Independent Data Analysis Centre

IEC:	Independent Ethics Committee
IgG:	Immunoglobulin G
IRB:	Institutional Review Board
iSRC:	internal Safety Review Committee
IU:	international unit
LAR:	Legally Acceptable Representative
LMIC:	Low- and Middle-Income Countries
LPS:	Lipopolysaccharide
LSLV:	Last Subject Last Visit
MedDRA:	Medical Dictionary for Regulatory Activities
OAg:	O-Antigen
OPK:	opsonophagocytic killing activity
PBMC:	peripheral blood mononuclear cell
PCR:	polymerase chain reaction
pDiary:	paper Diary
PP:	Project Physician
QDM:	Quantitative Decision Making
QTL:	quality tolerance limit
SADE:	Serious adverse device effect
SAE:	serious adverse event
SAP:	Statistical Analysis Plan
SBA:	serum bactericidal assay
SBIR:	System Built for Internet Randomisation
SDL:	Study Delivery Lead
SPM:	Study Procedures Manual
SRT:	Safety Review Team
SUSAR:	suspected unexpected serious adverse reaction
ULN:	upper limit of normal
USADE:	unanticipated serious adverse device effect
WHO:	World Health Organization
WOCBP:	Woman of Childbearing Potential
WRAIR:	Walter Reed Army Institute of Research

10.10.2. Glossary of terms

Adverse Drug Reaction:	***An adverse event where a causal relationship between a medicinal product and the adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.*** ***a. In the context of a clinical trial, an ADR can be serious or non-serious. Serious ADRs may be subject to expedited reporting if they are considered unexpected (see SUSAR definition).*** ***b. For marketed products, ADRs are subject to expedited reporting within the country where they are authorized***
Adverse event:	Any untoward medical occurrence in a patient or clinical investigation participant, 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 unfavourable 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.
Blinding:	A procedure in which 1 or more parties to the trial are kept unaware of the intervention 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 SAE. In an observer-blind study, the participant, the site, and sponsor personnel involved in the clinical evaluation of the participants are blinded while other study personnel may be aware of the treatment assignment.
Caregiver:	A ‘caregiver’ is someone who – lives in the close surroundings of a participant and has a continuous caring role or – has substantial periods of contact with a participant and is engaged in his/her daily health care (e.g., a relative of the participant, a nurse who helps with daily activities in case of residence in a nursing home).

	In the context of a clinical study, a caregiver could include an individual appointed to oversee and support the participant's compliance with protocol specified procedures.
Certified copy:	A copy (irrespective of the type of media used) of the original record that has been verified (i.e., by a dated signature or by generation through a validated process) to have the same information, including data that describe the context, content, and structure, as the original.
Challenge agents:	***A product given to trial participants to produce a physiological response that is necessary before the pharmacological action of the IMP can be assessed.***
Child in care:	A child who has been placed under the control or protection of an agency, organisation, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
Co-administered (concomitant) products:	***A product given to clinical trial participants as required in the protocol as part of their standard care for a condition which is not the indication for which the IMP is being tested and is therefore not part of the objective of the study.***
Combination product:	Combination product comprises any combination of – drug – device – biological product Each drug, device and biological product included in a combination product is a constituent part.
Comparator:	***Any product used as a reference (including placebo, marketed product, GSK or non-GSK) for an investigational product being tested in a clinical trial. This is any product that is being used to assess the safety, efficacy, or other measurable value against the test product (IMP).***
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.

Enrolment:	The process of registering a participant into a clinical study by assigning participant identification number after signing the ICF.
Essential documents:	Documents which individually and collectively permit evaluation of the conduct of a study and the quality of the data produced.
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the per-protocol analysis.
GSK Vaccines Institute for Global Health (GVGH):	A GSK-owned company, part of the GSK Global Health Programme, committed to develop vaccines for major neglected diseases of impoverished communities.
Immunological correlate of protection:	A correlate of risk that has been validated to predict a certain level of protection from the targeted endpoint.
Intervention:	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 participant.
Intervention number:	A number identifying an intervention to a participant, according to intervention allocation.
Invasive medical device:	A device which, in whole or in part, penetrates inside the body, either through a body orifice or through the surface of the body.
Investigational product:	***A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form***
Investigator:	A person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. The investigator can delegate trial-related duties and functions conducted at the trial site to qualified individual or party to perform those trial-related duties and functions.

Legally acceptable representative:	An individual, judicial or other body authorised under applicable law to consent on behalf of a prospective participant to the participant's participation in the clinical trial. The terms legal representative or legally authorised representative are used in some settings.
Medical device deficiency:	A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors and information supplied by the manufacturer.
Participant:	Term used throughout the protocol to denote an individual who has been contacted to participate or who participates in the clinical study as a recipient of the study intervention (vaccine(s)/product(s)/control). Synonym: subject
Participant number:	A unique identification number assigned to each participant who consents to participate in the study.
Pharmacogenomics:	The ICH E15 Guidance for Industry defines pharmacogenomics as the "Study of variation of DNA and RNA characteristics as related to drug or treatment response." Pharmacogenetics, 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 is not pharmacogenomics.
Placebo:	***An inactive substance or treatment that looks the same as, and is given in the same way as, an active drug or intervention/treatment being studied.***

Primary completion date:	The date that the final participant 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 prespecified protocol or was terminated.
Protocol amendment:	The ICH defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK further details this to include a change to an approved protocol that affects the safety of participants, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study.
Randomisation:	Process of random attribution of intervention to participants to reduce selection bias.
Remote visit:	This term refers to the visit conducted in the place other than the study site.
Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor and responsible for assuring proper conduct of clinical studies at 1 or more investigational sites.
Solicited event:	Events to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the participant or an observer during a specified follow-up period following study intervention administration.
Source data:	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).
Source documents:	Original legible documents, data, and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, participants' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, participant files, and records kept at the pharmacy, laboratories and at medico-technical departments involved in the clinical trial).

Study intervention:	Any investigational or marketed product(s) or placebo intended to be administered to a participant during the study.
Study monitor:	An individual assigned by the sponsor and responsible for assuring proper conduct of clinical studies at 1 or more investigational sites.
SUSAR:	***Suspected Unexpected Serious Adverse Reaction; in a clinical trial, a serious adverse reaction that is considered unexpected, i.e., the nature or severity of which is not consistent with the reference safety information (e.g., Investigator's Brochure for an unapproved investigational medicinal product). All adverse drug reactions (ADRs) that are both serious and unexpected are subject to expedited reporting.***
Telemedicine:	Telemedicine refers to the use of information technologies and electronic communications to provide clinical services to patients virtually. The digital transmission of medical imaging, virtual medical diagnosis and evaluations, and video consultations with specialists are all examples of telemedicine.
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.
Virtual visit:	This term refers to study visits conducted using multimedia or technological platforms.

10.11. Appendix 11: Protocol Amendment history

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents.

DOCUMENT HISTORY	
Document	**Date of Issue**
Protocol Final	26 May 2021
Protocol Amendment 1	23 September 2021
Protocol Amendment 2	14 February 2022
Protocol Amendment 3	7 February 2023
Protocol Amendment 4	31 Oct 2023

Detailed description of this Protocol Amendment:

Cover Page

The following comparator vaccine part of "Other study interventions" has been deleted:

- ~~Diphtheria, tetanus, and pertussis vaccine (INFANRIX)~~

The following comparator vaccine part of "Other study interventions" has been added:

- ***Diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis (inactivated), and Haemophilus influenzae type b vaccine (INFANRIX HEXA)***

Section 1.1 (Synopsis)

Under "Objectives and endpoints", the following tertiary objective has been modified:

- To measure natural exposure to *Shigella* in Stage 2 ***infant*** participants

Under "Objectives and endpoints", the following tertiary objective and corresponding endpoints have been modified:

• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ~~participants~~ ***adult, child, and infant safety cohorts,*** in ~~Europe~~***Africa (Stage ~~1~~2)***.	• Anti-serotype specific *Shigella* LPS* serum IgG GMTs, in adults 18 to 50 years of age in ~~Europe~~***Africa***, as measured by reference ELISA, before each vaccination (Day 1 and ***Day 85***/~~Day169~~***)***, ~~14 days after the first vaccination (Day 15),~~ and 28 days after each vaccination (Day 29 and Day 113~~/Day 197~~***)***. • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in children 24 to 59 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).***

	• ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in infants 9 months of age in the safety cohorts in Africa, as measured by reference ELISA, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ~~Europe~~ ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:800** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85***~~/Day 169~~***),*** ~~14 days after first vaccination (Day 15)~~ and 28 days after each vaccination (Day 29 and Day 113~~/Day 197~~***).*** • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ~~Europe~~***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:1600*** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85***~~Day 169~~***),*** ~~14 days after first vaccination (Day 15)~~ and 28 days after each vaccination (Day 29 and Day 113~~/Day 197~~***)***. • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested. **This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. ***This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

Section 1.3 (Schedule of Activities), Table 4 (Schedule of Activities for infants 9 months of age in Stage 2)

The following footnote in Table 4 has been modified:

[4]The control vaccination will consist of the ~~Infanrix~~***INFANRIX HEXA*** vaccine.

Section 2.3 (Benefit/Risk assessment)

The text in the second paragraph has been modified:

Detailed information about the known and expected benefits and risks, and expected AEs of MENVEO, BOOSTRIX, TYPHIM VI, MR-VAC, and ~~INFANRIX~~***INFANRIX HEXA*** can be found in the Prescribing Information/Summary of Product Characteristics.

Section 2.3.3 (Overall Benefit: Risk conclusion)

The text in the second paragraph has been modified:

The control vaccines administered to participants in this study as active comparators are widely used, and therefore have demonstrated medical benefit in the prevention of invasive meningococcal disease caused by *Neisseria meningitidis* groups A, C, W, Y (MENVEO), diphtheria, tetanus, and pertussis (~~INFANRIX,~~ BOOSTRIX), ***diphtheria/tetanus/pertussis/hepatitis B/polyomelitis (inactivated)/Haemophilus influenzae type b (INFANRIX HEXA)***, or typhoid fever caused by *Salmonella typhi* (TYPHIM VI).

Section 3 (Objectives and endpoints), Table 6 (Study objectives and endpoints)

Under "Objectives and endpoints", the following objective has been modified:

- To measure natural exposure to *Shigella* in Stage 2 ***infant*** participants

Under "Objectives and endpoints", the following objective and corresponding endpoints have been modified:

• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ~~participants~~ ***adult, child, and infant safety cohorts,*** in ~~Europe~~***Africa (Stage ~~1~~2)***.	• Anti-serotype specific *Shigella* LPS* serum IgG GMTs, in adults 18 to 50 years of age in ~~Europe~~***Africa***, as measured by reference ELISA, before each vaccination (Day 1 and ***Day 85***/~~Day169~~***),*** ~~14 days after the first vaccination (Day 15),~~ and 28 days after each vaccination (Day 29 and Day 113/~~Day 197~~***)***. • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in children 24 to 59 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in infants 9 months of age in the safety cohorts in Africa, as measured by reference ELISA, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ~~Europe~~ ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:800** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85***/~~Day 169~~***),*** ~~14 days after first vaccination (Day 15)~~ and 28 days after each vaccination (Day 29 and Day 113/~~Day 197~~***).*** • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).***

	• ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ~~Europe~~***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:1600*** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85***~~Day 169~~***),*** ~~14 days after first vaccination (Day 15)~~ and 28 days after each vaccination (Day 29 and Day 113~~/Day 197~~***)***. • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested. **This threshold (1:800, known as "Cohen's Threshold") is not validated but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. ***This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

Section 4.1 (Overall design), Table 10 (Study groups, intervention and blinding in infants in Stage 2)

In Table 10, text in the "Study intervention" column has been modified:

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 9 (observer-blind)
ST2_Infants_Control A_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ~~INFANRIX~~***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose A_Safety	10	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	•
ST2_Infants_Control B_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ~~INFANRIX~~***INFANRIX HEXA*** (Day 253)	•
ST2_Infants_Dose B_Safety	10	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	•

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 9 (observer-blind)
ST2_Infants_Control C_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), ~~INFANRIX~~***INFANRIX HEXA*** (Day 253)	●
ST2_Infants_Dose C_Safety	10	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	●
ST2_Infants_Control_Dose find	82	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 1 and Day 253), ~~INFANRIX~~***INFANRIX HEXA*** (Day 253)	●
ST2_Infants_Dose A_Dose find	82	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●
ST2_Infants_Dose B_Dose find	82	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●
ST2_Infants_Dose C_Dose find	82	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●

Section 4.2.3 (Rationale for Choice of control vaccines)

The text in the fourth paragraph has been modified:

For the infants in Africa, MENVEO and ~~INFANRIX~~***INFANRIX HEXA*** will be administered because the timing of administration matches their recommended administrations.

Section 6.1 (Study interventions administered), Table 11 (Study interventions administered)

In the table, the following column has been modified:

~~INFANRIX~~ ***INFANRIX HEXA***	
~~DT (≥ 30 IU) adsorbed on $Al(OH)_3$; TT (≥ 40 IU) adsorbed on $Al(OH)_3$; PT (25 µg) adsorbed on $Al(OH)_3$; FHA (25 µg) adsorbed on $Al(OH)_3$; PRN (8 µg) adsorbed on $Al(OH)_3$; $Al(OH)_3$ (0.5 mg Al^{3+}); Water for injections q.s. 0.5 Ml~~ ***DT[1] (≥ 30 IU); TT[1] (≥ 40 IU); PT (25 µg)[1]; FHA (25 µg)[1]; PRN (8 µg)[1]; HBsAg (10 µg)[2]; IPV1 (Mahoney strain) (40 DAgU); IPV2 (MEF-1***	***Hib (10 µg)-TT (~25 µg) adsorbed on $AlPO_4$; $AlPO_4$***

strain) (8 DAgU); IPV3 (Saukett strain) (32 DAgU); [1]adsorbed on $Al(OH)_3$ and [2]adsorbed on $AlPO_4$ (0.7 mg Al^{3+}); Water for injections	
~~Syringe, suspension for injection~~ ***Pre-filled syringe, suspension for suspension for injection***	***Vial, powder for suspension for injection***
~~control~~ ***Comparator***	
Combination Product	
Thigh	
Anterolateral	
1 in infants	
0.5 mL	
Refer to the Study Procedures Manual (SPM) for further details	
GSK	

In the table, the following row has been modified:

Study intervention name	AltSonflex1-2-3 Dose A (diluted from Dose C)	AltSonflex 1-2-3 Dose B (diluted from Dose C)	AltSonflex1-2-3 Dose C	altSonflex Placebo	MENVEO	BOOSTRIX	***INFANRIX HEXA***	TYPHIMVI	MR-VAC
Type	~~study~~***Investigational product***	~~study~~***Investigational product***	~~study~~***Investigational product***	~~control~~***Placebo***	~~control~~***Comparator***	~~control~~***Comparator***	***Comparator***	~~control~~***Comparator***	~~co-administration~~ ***Co-administered product***

Section 6.3.5 (Allocation of participants to assay subsets)

In the first bullet, the last sentence has been modified:

For this randomly selected subset of participants, available samples taken at Days 1, 15, 29, 85/169, and 113/197 will be tested to fulfil tertiary objectives (SBA, ***SBA against a panel of vaccine heterologous Shigella serotypes,*** systems serology analysis, and opsonophagocytosis assay).

Section 8.1.2 (Laboratory assays), Table 15 (Laboratory assays)

The text in a cell in the "Method" column from Table 15 has been modified:

Assay type	System	Component	Method	Laboratory*
Humoral Immunity (Antibody determination)	Serum	Anti-*S. sonnei* LPS/OAg total IgG	GVGH ELISA (Stage 1 secondary objective and Stage 2 primary and secondary objectives)	GVGH or GVGH designated laboratory
		Anti-*S. flexneri* 1b LPS/OAg total IgG		
		Anti-*S. flexneri* 2a LPS/OAg total IgG		
		Anti-*S. flexneri* 3a LPS/OAg total IgG		
		Bactericidal antibodies against *S. sonnei*	SBA	
		Bactericidal antibodies against *S. flexneri* 1b		
		Bactericidal antibodies against *S. flexneri* 2a		
		Bactericidal antibodies against *S. flexneri* 3a		
		Anti-*S. sonnei* LPS total IgG	Reference ELISA (Stage ~~1~~**2** tertiary objective)	WRAIR
		Anti-*S. flexneri* 1b LPS total IgG		
		Anti-*S. flexneri* 2a LPS total IgG		
		Anti-*S. flexneri* 3a LPS total IgG		
		Measles	ELISA (Stage 2 secondary objective)	GVGH designated laboratory
		Rubella		

Section 8.1.3 (Immunological read-outs), Table 16 (Immunological readouts)

Table 15 has been modified:

Blood sampling timepoint		Subset name (refer to Section 6.3.5)	No. participants	Component
Type of contact and timepoint	Sampling timepoint			
Adults in Europe (Stage 1) Group 1				
Visit 1 (Day 1), Visit 5 (Day 85)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~

Blood sampling timepoint		Subset name (refer to Section 6.3.5)	No. participants	Component
Type of contact and timepoint	**Sampling timepoint**			
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 113)	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Adults in Europe (Stage 1) Group 2				
Visit 1 (Day 1), Visit 5 (Day 169)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 197	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Adults in Africa (Stage 2)				
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	N/A	20	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-Vaccination	Stage 2 Adults	15	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri 1b*
				Bactericidal antibodies against *S. flexneri 2a*
				Bactericidal antibodies against *S. flexneri 3a*
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	N/A	20	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 1b* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 2a* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri 3a* LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	Stage 2 Adults	15	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri 1b*
				Bactericidal antibodies against *S. flexneri 2a*
				Bactericidal antibodies against *S. flexneri 3a*
Children in Africa (Stage 2)				
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	NA	40	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-Vaccination	Stage 2 Children	25	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	NA	40	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Anti-S. sonnei LPS total IgG (WRAIR)
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	Stage 2 Children	25	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Infants in Africa (Stage 2)-Safety cohort				
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	60	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
Visit 1 (Day 1)			***60***	***Anti-S. sonnei LPS total IgG (WRAIR)***

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 1 (Day 1)	Pre-vaccination	Stage 2 Infant Safety Cohort	40	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	Post-vaccination	N/A	60	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)			***60***	***Anti-S. sonnei LPS total IgG (WRAIR)***
				Anti-S. flexneri 1b LPS total IgG (WRAIR)
				Anti-S. flexneri 2a LPS total IgG (WRAIR)
				Anti-S. flexneri 3a LPS total IgG (WRAIR)
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	Post-vaccination	Stage 2 Infant Safety Cohort	40	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Infants in Africa (Stage 2)-Dose-finding cohort				
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	328	Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 1 (Day 1)				Measles IgG
				Rubella IgG
Visit 3 (Day 29), Visit 6 (Day 113), Visit 9 (Day 281)	Post-vaccination			Anti-*S. sonnei* LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 1b LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a LPS/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a LPS/OAg total IgG (GVGH)
				Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 9 (Day 281)				Measles IgG
				Rubella IgG

Section 8.2.2.1.2 (Stage 2 [Africa])

Under "Adults 18 to 50 years of age", the last sentence of the first paragraph has been modified:

[…] The subsequent 15 adults will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

Under "Children 24 to 59 months of age", the last sentence of the first and third paragraph has been modified:

[…] The subsequent 15 children will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

Under "Infants 9 months of age", the first, third, and fifth paragraphs have been modified:

After approval, 20 infants will receive 3 primary vaccinations of either Dose A of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ~~INFANRIX~~***INFANRIX HEXA*** for the third vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter.***

[…]

After approval, 20 infants will receive 3 primary vaccinations with either Dose B or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ~~INFANRIX~~***INFANRIX HEXA*** for the third vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

[…]

After approval, 20 infants will receive 3 primary vaccinations with either Dose C or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the third vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and ~~INFANRIX~~***INFANRIX HEXA*** for the third vaccination. The first 5 infants will then receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will ***start to*** be vaccinated in parallel ***at least 60 minutes thereafter***.

Section 8.2.2.3 (Study holding rules), Table 18 (Study holding rules)

In the table rows for Holding rules "2a" and "2b", under the column for "Safety cohorts", footnotes symbols (**) have been added:

Holding rule	Event	Number or percentage of participants needed to trigger a hold	
		Safety cohorts	Dose-finding cohorts
[…]			
2a	Any Grade 3 solicited administration-site event (lasting 48h or more) in an investigational group*, within the 7-days (Days 1-7) post-vaccination period	10%**	10%**
2b	Any Grade 3 unsolicited AE in an investigational group*, that can be reasonably attributed to the vaccination, within the 7-day (day 1-7) post-vaccination period or Any Grade 3 abnormality in prespecified haematological or biochemical laboratory parameters*** in an investigational group* within the 7-day (day 1-7) post-vaccination period	≥1 in all safety cohorts, except for ≥3 for Dose 2 data evaluation in Stage 1**	10%**

The following table footnote has been modified:

In-stream ***and planned* blinded monitoring will evaluate the total number of participants vaccinated at the time of the safety evaluation (candidate vaccine and control recipients) and will trigger an *ad hoc* iSRC if the percentage threshold is reached.[…].

Section 9.4.3 (Tertiary endpoints)

Under "Statistical Analysis Methods", the first sentence has been modified:

For participants in ~~Europe~~***Africa in the safety cohorts***, for each sampling timepoint, and for each antigen the following statistics will be computed, as measured by reference ELISA:

[…]

Section 9.5.1 (Sequence of analyses)

A sentence has been added to the last paragraph of the section:

Data for tertiary endpoints which are available at the time of planned analyses will be included in the interim analyses. Data for tertiary endpoints that become available at later timepoints will be documented in annex(es) to the final CSR or in an integrated final CSR.

Section 10.2 (Appendix 2: Clinical laboratory tests)

Under "Anti-*Shigella* serotype-specific antibody determination from serum", the first sentence of the second paragraph has been modified:

Anti-*Shigella* serotype-specific IgG antibodies in serum will also be measured by ELISA in the WRAIR laboratories (only for sera from ~~Stage 1~~ ***the safety cohorts in adults, children, and infants in Stage 2 up to 28 days after their third vaccination***), based on a method previously reported [Riddle, 2016]. […].

Section 10.2.1 (Protocol required safety laboratory assessments), Table 27 (Protocol required safety laboratory assessments)

In the table row for "Infants in Africa (Stage 2)" and within "Clinical chemistry", the following parameters have been added:

Infants in Africa (Stage 2)	**Haematology**	• Leukocytes (WBC) • Erythrocytes (RBC) • Haemoglobin • Haematocrit • Platelets • Eosinophils • Basophils • Neutrophils • Monocytes • Lymphocytes
	Clinical chemistry	• Creatinine • ***Blood urea*** • ***Sodium*** • ***Potassium*** • AST • ALT

Section 10.8.4 (Stage 2 = Infants [Africa])

In the table, the "Creatinine" row has been modified:

Laboratory test	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially life-threatening (Grade 4)**
Creatinine ***– µmol/L***	~~≥1.1 – 1.3 x ULN~~ ***≥40.7 – 48.1***	~~>1.3 to 1.8 x ULN OR Increase to 1.3 to <1.5 x participant's baseline~~ ***>48.1 – 66.6***	~~>1.8 to <3.5 x ULN OR Increase to 1.5 to <2.0 x participant's baseline~~ ***>66.6 – <129.5***	~~≥3.5 x ULN OR Increase of ≥2.0 x participant's baseline~~ ***≥129.5***

Section 10.10.2 (Glossary of terms)

The following terms and their definitions have been added:

Adverse Drug Reaction:	***An adverse event where a causal relationship between a medicinal product and the adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.*** ***a. In the context of a clinical trial, an ADR can be serious or non-serious. Serious ADRs may be subject to expedited reporting if they are considered unexpected (see SUSAR definition).*** ***b. For marketed products, ADRs are subject to expedited reporting within the country where they are authorized***

[…]

Challenge agents:	***A product given to trial participants to produce a physiological response that is necessary before the pharmacological action of the IMP can be assessed.***

[…]

Co-administered (concomitant) products:	***A product given to clinical trial participants as required in the protocol as part of their standard care for a condition which is not the indication for which the IMP is being tested and is therefore not part of the objective of the study.***

[…]

Comparator:	***Any product used as a reference (including placebo, marketed product, GSK or non-GSK) for an investigational product being tested in a clinical trial. This is any product that is being used to assess the safety, efficacy, or other measurable value against the test product (IMP).***

[…]

Investigational product:	***A pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form***

[…]

Placebo:	***An inactive substance or treatment that looks the same as, and is given in the same way as, an active drug or intervention/treatment being studied.***

[…]

SUSAR:	***Suspected Unexpected Serious Adverse Reaction; in a clinical trial, a serious adverse reaction that is considered unexpected, i.e., the nature or severity of which is not consistent with the reference safety information (e.g., Investigator's Brochure for an unapproved investigational medicinal product). All adverse drug reactions (ADRs) that are both serious and unexpected are subject to expedited reporting.***

Section 11 (References)

The following references have been added:

BOOSTRIX(Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, adsorbed). Product information. February 2023.

[…]

INFANRIX HEXA (Diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis (inactivated), and Haemophilus influenzae type b vaccine). Product information. May 2023.

[…]

MENVEO (Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM197 Conjugate Vaccine). Product information. July 2023.

[…]

MR-VAC (Measles and rubella vaccine [live]). Product information. December 2021.

[…]

TYPHIM VI. Salmonella typhi Vi Capsular Polysaccharide Vaccine. Product information. October 2021.

Detailed description of Protocol Amendment 3:

Section 1.1 (Synopsis)

Under "Objectives and endpoints", the following primary endpoint has been modified:

- Anti-serotype specific *Shigella* lipopolysaccharide (LPS)/***O-Antigen (OAg)****** serum immunoglobulin G (IgG) ~~titres~~ ***geometric mean concentrations (GMC),*** as measured by ~~reference~~ ***GVGH*** enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the third vaccination (Day 281).

Under "Objectives and endpoints", the following secondary endpoints have been modified:

- Anti-serotype specific *Shigella* LPS/***OAg****** serum IgG ~~geometric mean titres (GMTs)~~***GMCs***, in adults 18 to 50 years of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Anti-serotype specific *Shigella* LPS/***OAg****** serum IgG ~~GMTs~~***GMCs***, in children 24 to 59 months of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Anti-serotype specific *Shigella* LPS/***OAg****** serum IgG ~~GMTs~~***GMCs***, in infants 9 months of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197).
- Number of adults in Africa 18 to 50 years of age achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of children 24 to 59 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/***OAg*** in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of infants 9 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197).
- Number of adults in Africa 18 to 50 years of age achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of children 24 to 59 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/***OAg*** in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of infants 9 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/***OAg***, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Number of adults 18 to 50 years of age in Africa showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/***OAg*** * serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85).
- Number of children 24 to 59 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/***OAg*** * serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85).
- Number of infants 9 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/***OAg****** serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29, Day 113, and Day 281), compared to baseline (Day 1, Day 85, and Day 253).

Under "Objectives and endpoints", the following footnotes under secondary endpoints have been modified:

*This threshold (1:800, known as "Cohen's Threshold") ***is not validated but*** has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] ~~and~~***. It*** will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the Statistical Analysis Plan (SAP).
This ***non-validated threshold* (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

Under "Objectives and endpoints", the following tertiary objective have been modified:

- To evaluate the immune response induced by the ***alt***Sonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1)***, subsets of adults, children,*** and ~~25% of~~ infants ***in the safety cohorts, and all infants in the dose-finding cohorts,*** ~~9 months of age~~ in Africa (Stage 2)~~, in the dose-finding cohort~~, using the serum bactericidal assay (SBA).
- To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and ~~25%~~***a subset*** of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.
- To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ~~all~~ participants in Europe (Stage 1).

Under "Objectives and endpoints", the following tertiary endpoints have been modified:

- Anti-serotype specific* serum bactericidal antibody GMTs in ~~25% of~~ ***all*** infants 9 months of age in the dose-finding cohort, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).

Under "Objectives and endpoints", the following tertiary objective has been added:

- ***To measure natural exposure to Shigella in Stage 2 participants***

Under "Objectives and endpoints", the following tertiary endpoints have been added:

- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of adults in Africa 18 to 50 years of age, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113)***
- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of children 24 to 59 months of age before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113)***
- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of infants 9 months of age in the safety cohort, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29, Day 113, and Day 281)***
- ***Anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA***

Under "Objectives and endpoints", the following footnotes under tertiary endpoints have been modified:

This threshold (1:800, known as "Cohen's Threshold") ***is not validated but* has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] ~~and~~***. It*** will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.
This ***non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

Section 1.2 (Schema)

In the fourth schema depicting the study design for the "Infants 9 months of age", the legend to the dotted vertical line between Day 113 and Day 253 was modified as follows:

"Interim analysi~~s~~***es*** ~~in dose-finding cohorts~~"

Section 1.3 (Schedule of Activities), Table 2 (Schedule of Activities for Adults 18 to 50 years of age in Stage 2)

The following footnote has been deleted:

~~Note: The double-line borders following Day 113 indicate final analyses in these participants, which will be performed on all data obtained up to Day 113.~~

Section 1.3 (Schedule of Activities), Table 3 (Schedule of Activities for Children 24 to 59 months of age in Stage 2)

The following footnote has been deleted:

~~Note: The double-line borders following Day 113 indicate final analyses in these participants, which will be performed on all data obtained up to Day 113.~~

Section 1.3 (Schedule of Activities), Table 4 (Schedule of Activities for Infants 9 months of age in Stage 2)

The following footnote has been modified:

Note: The double-line borders following Day 113 indicate interim analyses ***in the infant safety cohort (interim analysis 3) and in*** the infant dose-finding cohort ***(interim analysis 4)***, which will be performed on all data obtained up to Day 113. The double-line borders following Day 281 indicate ~~interim analysis in the infant safety cohorts and~~ final analysis ***of the study***, which will be performed on all ***study*** data obtained up to Day 281.

Section 3 (Objectives and Endpoints), Table 6 (Study objectives and endpoints)

The following primary endpoint has been modified:

- Anti-serotype specific *Shigella* lipopolysaccharide (LPS)***/O-Antigen (OAg)****** serum immunoglobulin G (IgG) ~~titres~~ ***geometric mean concentrations (GMC),*** as measured by ~~reference~~ ***GVGH*** enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the third vaccination (Day 281).

The following secondary endpoints have been modified:

- Anti-serotype specific *Shigella* LPS***/OAg****** serum IgG ~~geometric mean titres (GMTs)~~***GMCs***, in adults 18 to 50 years of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Anti-serotype specific *Shigella* LPS***/OAg****** serum IgG ~~GMTs~~***GMCs***, in children 24 to 59 months of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Anti-serotype specific *Shigella* LPS***/OAg****** serum IgG ~~GMTs~~***GMCs***, in infants 9 months of age in Africa, as measured by ~~reference~~***GVGH*** ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).

- Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197).
- Number of adults in Africa 18 to 50 years of age achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of children 24 to 59 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS***/OAg*** in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of infants 9 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197).
- Number of adults in Africa 18 to 50 years of age achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of children 24 to 59 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS***/OAg*** in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113).
- Number of infants 9 months of age in Africa achieving a ~~reference~~***GVGH*** ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS***/OAg***, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Number of adults 18 to 50 years of age in Africa showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS***/OAg*** * serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85).
- Number of children 24 to 59 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS***/OAg*** * serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85).
- Number of infants 9 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS***/OAg**** serum IgG ~~titres~~***concentrations***, as measured by ~~reference~~***GVGH*** ELISA, at 28 days after each vaccination (Day 29, Day 113, and Day 281), compared to baseline (Day 1, Day 85, and Day 253).

The following footnotes under secondary endpoints have been modified:

*This threshold (1:800, known as "Cohen's Threshold") ***is not validated but*** has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]~~ and~~***. It*** will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.
This ***non-validated* threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

The following tertiary objective has been added:

- ***To measure natural exposure to Shigella in Stage 2 participants.***

The following tertiary endpoints have been added:

- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of adults in Africa 18 to 50 years of age, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113).***

- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of children 24 to 59 months of age before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113).***
- ***Anti-serotype specific* serum bactericidal antibody GMTs in a subset of infants 9 months of age in the safety cohort, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29, Day 113, and Day 281).***
- ***Anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA.***

The following tertiary objective have been modified:

- To evaluate the immune response induced by the ***alt***Sonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1)***, subsets of adults, children,*** and ~~25% of~~ infants ***in the safety cohorts, and all infants in the dose-finding cohorts,*** ~~9 months of age~~ in Africa (Stage 2)~~, in the dose-finding cohort~~, using the serum bactericidal assay (SBA).
- To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and ~~25%~~***a subset*** of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.
- To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ~~all~~ participants in Europe (Stage 1).

The following tertiary endpoints have been modified:

- Anti-serotype specific* serum bactericidal antibody GMTs in ~~25% of~~ ***all*** infants 9 months of age in the dose-finding cohort, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).

The following footnotes under tertiary endpoints have been modified:

This threshold (1:800, known as "Cohen's Threshold") ***is not validated but* has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]~~ and~~***. It*** will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.
This ***non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

Section 4.1 (Study design), Figure 1 (Study design overview)

In the fourth schema depicting the study design for the "Infants 9 months of age", the legend to the dotted vertical line between Day 113 and Day 253 was modified as follows:

"Interim analysi~~s~~***es*** ~~in dose-finding cohorts~~"

Section 6.3.5 (Allocation of participants to assay subsets)

The following bullets have been added:

- ***Stage 2 Adults Subset: all participants from the adult altSonflex1-2-3 group in Stage 2 ("ST2_Adults_Dose C") and a random sample of 50% of participants from the adult control study group in Stage 2 ("ST2_Adults_Control C") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, and 113.***

- ***Stage 2 Children Subset: all participants from children altSonflex1-2-3 groups in Stage 2 ("ST2_Children_Dose B" and "ST2_Children_Dose C") and a random sample of 25% participants from the children control study groups in Stage 2 ("ST2_Children_Control B" and "ST2_Children_Control C") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, and 113.***
- ***Stage 2 Infants Safety Cohort Subset: all participants from the infant altSonflex1-2-3 safety cohort groups ("ST2_Infants_Dose A_Safety", "ST2_Infants_Dose B_Safety", and "ST2_Infants_Dose C_Safety") and a random sample of 33.33% participants from the infant safety cohort control study groups in Stage 2 ("ST2_Infants_Control A_Safety", "ST2_Infants_Control B_Safety", and "ST2_Infants_Control C_Safety") will be selected. For the selected subset of participants, SBA will be performed on available samples taken at Days 1, 29, 113, and 281.***

The following bullet has been modified:

- Stage 2 Infants Dose-Finding Cohort Subset: ~~25% of participants will be randomly selected from each infant study intervention study group of the dose-finding cohort ("ST2_Infants_Control_Dose find", "ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose B_Dose find", and "ST2_Infants_Dose C_Dose find"). For this randomly selected subset of participants, all available samples taken at Days 1, 29, 85, 113, 253, and 281 will be tested to fulfil tertiary objectives (SBA, systems serology analysis, and opsonophagocytosis assay).~~ ***a subset of participants from the infant dose-finding cohorts ("ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose B_Dose find", and "ST2_Infants_Dose C find") will be randomly selected. For this randomly selected subset of participants, systems serology analysis, SBA against a panel of vaccine heterologous Shigella serotypes, and opsonophagocytosis assay will be performed on available samples to fulfil tertiary objectives.***

Section 8 (Study assessments and procedures)

The following bullet has been modified:

- If despite best efforts, it is not possible to perform the Visits within the interval predefined in Table 5 of the protocol ***(e.g., due to COVID pandemic restrictions or potential delays in releasing outcomes of safety reviews)***, then the interval may be extended for the specified Visits up to a maximum length of days as presented in Table 13.

Section 8 (Study assessments and procedures), Table 13 (Intervals between study visits during special circumstances)

The allowed interval ranges between Visit 3 and Visit 4 in Stage 2 have been modified as follows:

Stage 2 - Adults 18 to 50 years of age		
Visit 3→Visit 4	56 days	50 days-~~64~~***70*** days
Stage 2 - Children 24 to 59 months of age		
Visit 3→Visit 4	56 days	50 days-~~64~~***70*** days
Stage 2 - Infants 9 months of age		
Visit 3→Visit 4	56 days	50 days-~~64~~***70*** days

Section 8.1.1 (Biological samples)

The following bullet has been modified:

- Children 24 to 59 ~~years~~***months*** of age in Stage 2: ~74 mL

Section 8.1.2 (Laboratory assays), Table 15 (Laboratory assays)

The table has been modified as follows:

Assay type	System	Component	Method	Laboratory*
Humoral Immunity (Antibody determination)	Serum	Anti-*S. sonnei* LPS/***OAg*** total IgG	GVGH ELISA (Stage 1 secondary objectives ***and Stage 2 primary and secondary objectives***)	GVGH or GVGH designated laboratory
		Anti-*S. flexneri* 1b ***LPS***/OAg total IgG		
		Anti-*S. flexneri* 2a ***LPS***/OAg total IgG		
		Anti-*S. flexneri* 3a ***LPS***/OAg total IgG		
		Bactericidal antibodies against *S. sonnei*	SBA	
		Bactericidal antibodies against *S. flexneri* 1b		
		Bactericidal antibodies against *S. flexneri* 2a		
		Bactericidal antibodies against *S. flexneri* 3a		
		~~Anti-*S. sonnei* LPS total IgG~~	~~Reference ELISA (Stage 2 primary and secondary objectives)~~	WRAIR
		~~Anti-*S. flexneri* 1b LPS total IgG~~		
		~~Anti-*S. flexneri* 2a LPS total IgG~~		
		~~Anti-*S. flexneri* 3a LPS total IgG~~		
		Anti-*S. sonnei* LPS total IgG	Reference ELISA (Stage 1 tertiary objective)	
		Anti-*S. flexneri* 1b LPS total IgG		
		Anti-*S. flexneri* 2a LPS total IgG		
		Anti-*S. flexneri* 3a LPS total IgG		
		Measles	ELISA (Stage 2 secondary objective)	***GVGH designated laboratory***
		Rubella		

Section 8.1.2 (Laboratory assays)

The following sentence has been modified:

Exploratory systems serology analysis assays, panel SBA, ~~and the~~ opsonophagocytosis assays, ***and anti-Shigella conserved protein IgG ELISA assays*** will be performed by the GVGH laboratory or a laboratory designated by GVGH.

Section 8.1.3 (Immunological read-outs), Table 16 (Immunological read-outs)

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
Adults in Europe (Stage 1) Group 1				
Visit 1 (Day 1), Visit 5 (Day 85)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/***OAg*** total IgG (GVGH)
				Anti-*S. flexneri* 1b ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. sonnei* LPS total IgG (WRAIR)
				Anti-*S. flexneri* 1b LPS total IgG (WRAIR)
				Anti-*S. flexneri* 2a LPS total IgG (WRAIR)
				Anti-*S. flexneri* 3a LPS total IgG (WRAIR)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 113)	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/***OAg*** total IgG (GVGH)
				Anti-*S. flexneri* 1b ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. sonnei* LPS total IgG (WRAIR)
				Anti-*S. flexneri* 1b LPS total IgG (WRAIR)
				Anti-*S. flexneri* 2a LPS total IgG (WRAIR)
				Anti-*S. flexneri* 3a LPS total IgG (WRAIR)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Adults in Europe (Stage 1) Group 2				
Visit 1 (Day 1), Visit 5 (Day 169)	Pre-Vaccination	N/A	51	Anti-*S. sonnei* LPS/***OAg*** total IgG (GVGH)
				Anti-*S. flexneri* 1b ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. sonnei* LPS total IgG (WRAIR)
				Anti-*S. flexneri* 1b LPS total IgG (WRAIR)
				Anti-*S. flexneri* 2a LPS total IgG (WRAIR)

Blood sampling timepoint		Subset name (refer to Section 6.3.5)	No. participants	Component
Type of contact and timepoint	**Sampling timepoint**			
				Anti-*S. flexneri* 3a LPS total IgG (WRAIR)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 3 (Day 15), Visit 4 (Day 29), Visit 7 (Day 197	Post-Vaccination	N/A	51	Anti-*S. sonnei* LPS/***OAg*** total IgG (GVGH)
				Anti-*S. flexneri* 1b ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 2a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. flexneri* 3a ***LPS***/OAg total IgG (GVGH)
				Anti-*S. sonnei* LPS total IgG (WRAIR)
				Anti-*S. flexneri* 1b LPS total IgG (WRAIR)
				Anti-*S. flexneri* 2a LPS total IgG (WRAIR)
				Anti-*S. flexneri* 3a LPS total IgG (WRAIR)
		25% Stage 1 Adults	13	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Adults in Africa (Stage 2)				
~~Visit 1 (Day 1), Visit 4 (Day 85)~~	~~Pre-Vaccination~~	~~N/A~~	~~20~~	~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
~~Visit 3 (Day 29), Visit 6 (Day 113)~~	~~Post-vaccination~~			~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	N/A	20	Anti-*S. sonnei* LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (**GVGH**)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (**GVGH**)
Visit 1 (Day 1)	***Pre-Vaccination***	***Stage 2 Adults***	***15***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	N/A	20	Anti-*S. sonnei* LPS/***OAg*** total IgG ***(GVGH)***
				Anti-*S. flexneri* 1b LPS/**OAg** total IgG ***(GVGH)***
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG ***(GVGH)***
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG ***(GVGH)***
Visit 3 (Day 29), Visit 6 (Day 113)	***Post-vaccination***	***Stage 2 Adults***	***15***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Children in Africa (Stage 2)				
~~Visit 1 (Day 1), Visit 4 (Day 85)~~	~~Pre-Vaccination~~	~~NA~~	~~40~~	~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
~~Visit 3 (Day 29), Visit 6 (Day 113)~~	~~Post-vaccination~~			~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~

Blood sampling timepoint		**Subset name** (refer to Section 6.3.5)	**No. participants**	**Component**
Type of contact and timepoint	**Sampling timepoint**			
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
Visit 1 (Day 1), Visit 4 (Day 85)	Pre-Vaccination	NA	40	Anti-*S. sonnei* LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (***GVGH***)
Visit 1 (Day 1)	***Pre-Vaccination***	***Stage 2 Children***	***25***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Visit 3 (Day 29), Visit 6 (Day 113)	Post-vaccination	NA	40	Anti-*S. sonnei* LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (***GVGH***)
Visit 3 (Day 29), Visit 6 (Day 113)	***Post-vaccination***	***Stage 2 Children***	***25***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Infants in Africa (Stage 2)-Safety cohort				
~~Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)~~	~~Pre-vaccination~~	~~N/A~~	~~60~~	~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
~~Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)~~	~~Post-vaccination~~		~~60~~	~~Anti-*S. sonnei* LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 1b LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 2a LPS total IgG (WRAIR)~~
				~~Anti-*S. flexneri* 3a LPS total IgG (WRAIR)~~
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	60	Anti-*S. sonnei* LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (***GVGH***)
Visit 1 (Day 1)	***Pre-vaccination***	***Stage 2 Infant Safety Cohort***	***40***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	Post-vaccination	N/A	60	Anti-*S. sonnei* LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (***GVGH***)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (***GVGH***)
Visit 3 (Day 29), Visit 6 (Day 113), and Visit 9 (Day 281)	***Post-vaccination***	***Stage 2 Infant Safety Cohort***	***40***	***Bactericidal antibodies against S. sonnei***
				Bactericidal antibodies against S. flexneri 1b
				Bactericidal antibodies against S. flexneri 2a
				Bactericidal antibodies against S. flexneri 3a
Infants in Africa (Stage 2)-Dose-finding cohort				
Visit 1 (Day 1), Visit 4 (Day 85), and Visit 7 (Day 253)	Pre-vaccination	N/A	328	Anti-*S. sonnei* LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)

Blood sampling timepoint		Subset name (refer to Section 6.3.5)	No. participants	Component
Type of contact and timepoint	**Sampling timepoint**			
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
		~~25% Dose-Finding Cohort~~	~~82~~	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 1 (Day 1)		N/A	328	Measles IgG
				Rubella IgG
Visit 3 (Day 29), Visit 6 (Day 113), Visit 9 (Day 281)	Post-vaccination	N/A	328	Anti-*S. sonnei* LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
				Anti-*S. flexneri* 1b LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
				Anti-*S. flexneri* 2a LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
				Anti-*S. flexneri* 3a LPS/***OAg*** total IgG (~~WRAIR~~ ***GVGH***)
		~~25% Dose-Finding Cohort~~	~~82~~	Bactericidal antibodies against *S. sonnei*
				Bactericidal antibodies against *S. flexneri* 1b
				Bactericidal antibodies against *S. flexneri* 2a
				Bactericidal antibodies against *S. flexneri* 3a
Visit 9 (Day 281)		N/A	328	Measles IgG
				Rubella IgG

Section 8.1.6 (Immunological correlates of protection)

The following sentence has been added to the first paragraph:

No generally accepted immunological correlate of protection (CoP) has been demonstrated so far for the antigens used in the candidate vaccine. ***For S. sonnei, the GVGH ELISA level equivalent threshold values of 1:800 and 1:1600 to Cohen thresholds are not externally validated [Cohen, 2022], and have been chosen for the purpose of comparison to previous GVGH studies.***

Section 8.2.1.1 (Collection of demographic data)

The following sentences have been added to the paragraph:

Record demographic data such as date of birth (month and year only for adults 18 to 50 years of age; day, month, and year for children 18 to 59 months of age and infants 9 months of age), sex, and race in the participant's eCRF. ***Collection of sex and race data is necessary to assess and monitor the diversity of the trial participants, and to determine if the trial participants are truly representative of the impacted population. Date of birth is collected to stratify the population and determine the impact of the study intervention by age. For infants, the date of birth is also used to ensure that participants are eligible for inclusion in the study.***

Section 8.2.2 (Study holding rules and safety monitoring)

The following paragraph has been added at the end of the Section:

If a safety concern is identified during safety monitoring that may change the benefit-risk profile of 1 or more dose levels, appropriate actions will be taken. This may include termination of administration of 1 or more dose levels and enrolment with approximately 82 participants per group only for the remaining dose level(s) that would continue to be evaluated, in the dose-finding cohort and/or the corresponding dose-escalation steps.

Section 8.2.2.2 (Outcome of safety evaluation)

The following bullet has been modified:

- If no safety signal***/concern*** is observed, the favourable outcome of the safety evaluations will be documented and provided in writing, authorising the investigator to start vaccination of participants with the subsequent dose as well as enrolment and vaccination of the remaining participants of the next step of the study.

Section 8.3.3 (Regulatory reporting requirements for SAEs, pregnancies, and other events), Table 24 (Contact information for reporting SAEs, pregnancies and study holding rules)

In the lower left box for "Back up study contact for reporting SAEs and pregnancies", the email address and fax number have been modified:

Email address: ~~Rix.CT-safety-vac@gsk.com~~ ***ogm28723@gsk.com***

Fax: +32 2 656 51 16 ~~or +32 2 656 80 09~~

Section 9.2.2 (Stage 2 Dose-finding cohort)

The following sentence in the second paragraph has been modified:

Immunogenic responses will be measured by ELISA as ~~GMT~~***GMC*** and two-sided 95% Confidence Intervals (CI) of anti-serotype specific *Shigella* LPS/OAg serum IgG for all 4 serotypes (*S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a).

The paragraph immediately before Figure 3 has been modified:

The rationale for the selection strategy used to identify the preferred dose is that in the absence of an appreciable increment in response between different doses (above defined as 2-fold increase in ratio of ~~GMT~~***GMC***), the preferred formulation is the one with lowest amount of OAg. The sample size of 82 infants per group has been calculated according to scenarios 3, 4, and 5 where at least one t-test is performed to select the preferred dose. ***In case a dose level is discontinued due to safety concerns, at most 1 t-test comparing higher to lower dose will be performed to select the preferred dose.*** If scenarios 1 or 2 occur, no statistical test will be performed, and Dose A will be selected as the preferred one.

The sentence immediately after Figure 3 has been modified:

Table 25 shows the power achieved with the target sample size (72 evaluable participants per group) to demonstrate a 2-fold increase in ratio of ~~GMT~~***GMC***.

The following bullet at the end of the Section has been modified:

- Minimum Detectable Difference between groups: 0.301 on a log10 scale, equivalent to a 2-fold difference in ratios of ~~GMTs~~***GMCs***.

Section 9.4.1 (Primary endpoints)

Under "Statistical analysis method" related to "Primary immunogenicity", the following content has been modified or added:

Within-groups assessment
For each study group (Dose A, Dose B, Dose C, and control vaccine) of the 9-month infant dose-finding cohort, the ~~GMT~~***GMC*** with 95% CI of anti-serotype specific *Shigella* LPS***/OAg**** total IgG as measured by ~~reference~~***GVGH*** ELISA will be calculated at 28 days after third vaccination.
Between groups assessment
For each of the 4 serotypes in 9-month infant dose-finding cohort, the preferred dose at 28 days after third vaccination will be identified according to the scenarios reported in Figure 3:

- Scenario 1 and 2: Dose A
- Scenario 3: t-test comparing Dose C with Dose A
- Scenario 4: t-test comparing Dose B with Dose A
- Scenario 5: hierarchical sequential with the 3 following tests:
  1) t-test comparing Dose C with Dose A: if null hypothesis is rejected then
  2) t-test comparing Dose B with Dose A: if null hypothesis is rejected then
  3) t-test comparing Dose B with Dose C.

In addition to scenarios reported in Figure 3, in case of a dose level discontinuation

- ***if the lower dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then the lower dose will be selected as the preferred dose without performing any statistical test***
- ***if the higher dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then a t- test comparing the higher with lower dose will be performed to select the preferred dose***

Unadjusted Ratios of ~~GMTs~~***GMCs*** with 95% CI between groups for all pairwise comparisons will be calculated.
**S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.

Section 9.4.2 (Secondary endpoints)

Under "Statistical analysis method" related "Secondary immunogenicity", the following bullets have been modified:

Within-groups assessment
For each study group in participants in Europe, for each sampling timepoint, and for each antigen, the following statistics will be computed:

- Unadjusted GMCs with 95% CI of anti-serotype specific *Shigella* LPS/OAg* total IgG as measured by GVGH ELISA
- Within-participant geometric mean ratios (GMRs) will be computed for ~~GMTs~~***GMCs*** with 95% CI at each time point after vaccination versus baseline (Day 1)
- Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by GVGH ELISA

For each study group in participants in Africa, for each sampling timepoint, and for each antigen, the following statistics will be computed:

- Unadjusted ~~GMTs~~***GMCs*** with 95% CI of anti-serotype specific *Shigella* LPS***/OAg**** total IgG as measured by ~~reference~~***GVGH*** ELISA

- Within-participant GMRs will be computed for ~~GMTs~~***GMCs*** with 95% CI at each time point after vaccination versus baseline (Day 1)
- Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by ~~reference~~***GVGH*** ELISA

Section 9.4.3 (Tertiary endpoints)

Under "Statistical analysis method" related to "Tertiary immunogenicity", the following content has been modified and text added:

For participants in Europe, for each sampling timepoint, and for each antigen the following statistics will be computed, as measured by reference ELISA:

- Unadjusted GMTs with 95% CI of anti-serotype specific *Shigella* LPS/O~~a~~***A***g* total IgG
- Within-participant GMRs will be computed for GMTs with 95% CI at each time point after vaccination versus baseline (Day 1)
- Number and percentage of participants with 1:800 and 1:1600 equivalent titres with exact 95% CI

~~From the subset defined by 25% randomly chosen participants from the 9-month infant dose-finding cohort, the following statistics will be computed:~~

- ~~Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before and 28 days after each vaccination~~

From the subset defined by 25% randomly chosen participants from the Stage 1 adults 18 to 50 years of age, ***"Stage 1 Adults Subset",*** the following statistics will be computed:

- Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before, 15 days after first vaccination, and 28 days after each vaccination

From the subset defined by randomly chosen participants from the Stage 2 adults 18 to 50 years of age ("Stage 2 Adults Subset"), the following statistics will be computed:

- ***Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination***

From the subset defined by randomly chosen participants from the Stage 2 children 24 to 59 months of age ("Stage 2 Children Subset"), the following statistics will be computed:

- ***Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination***

From the subset defined by randomly chosen participants from the Stage 2 infant 9 month of age safety cohort ("Stage 2 Infant Safety Cohort Subset"), the following statistics will be computed:

- ***Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination***

For all participants in the 9-month infant dose-finding cohort, the following statistics will be computed

- ***Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before and 28 days after each vaccination***

For all the above, within-participant GMRs will be computed for GMT with 95% CI at each time point after vaccination versus baseline (Day 1). Additional exploratory immunology including system serology analysis, panel SBA, ~~and~~ opsonophagocytosis assay***s***, ***and anti-Shigella conserved protein IgG ELISA assays*** will be performed on the aforementioned subsets of 25% randomly chosen ***Stage 1 adult*** participants and ***on the infant dose-finding cohort. Results*** will be reported by group and timepoint using descriptive summary statistics. This analysis will be described in a dedicated SAP.

Section 9.5 (Interim analyses)

The following sentence has been modified:

To accelerate evaluation of vaccine immunogenicity in the target population and facilitate planning of future studies, ~~3~~***4***-study interim analyses and 1 final analysis (Stage 1 and Stage 2) will be performed as described in Section 9.5.1.

Section 9.5.1 (Sequence of analyses)

The following paragraph has been added:

c. Stage 2 interim analysis at Day 113 on immunogenicity data from all infant safety cohorts (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 safety cohorts, in order to obtain early immunogenicity data analysis and facilitate planning for subsequent clinical development, to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

The following paragraph has been modified:

~~c.~~***d.*** Stage 2 interim analysis at Day 113 on immunogenicity data from infant dose-finding cohort (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 cohorts, ~~in order to obtain early immunogenicity data analysis~~ ***to facilitate planning for subsequent clinical development***; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

Section 10.2 (Appendix 2: Clinical laboratory tests)

Under "Anti-*Shigella* serotype-specific antibody determination from serum", the following paragraph has been moved up and modified:

Anti-*Shigella* serotype-specific IgG antibodies in serum will be also measured by an ELISA assay developed by the GVGH laboratories (Siena, Italy), based on a method previously reported [Launay, 2017; Launay, 2019]. The assay will be performed in a laboratory designated by GVGH. The ELISA is performed using *S. sonnei,* ~~LPS or~~ *S. flexneri* 1b, ***S.flexneri*** 2a or ***S. flexneri*** 3a ***LPS/***O***A***g as coating antigen. For this assay, a standard curve is run in duplicate on each plate. Results are expressed in ELISA units (EU)/mL of serum. One unit equals the reciprocal of the dilution to give an optical density of 1.

Under "Anti-*Shigella* serotype-specific antibody determination from serum", The following paragraph has been modified:

Anti-*Shigella* serotype-specific IgG antibodies in serum will ***also*** be measured by ELISA in the WRAIR laboratories ***(only for sera from Stage 1)***, based on a method previously reported [Riddle, 2016]. The ELISA is performed using *S. sonnei* ~~or~~***,*** *S. flexneri* 1b, ***S. flexneri*** 2a***,*** or ***S. flexneri*** 3a LPS as coating antigen. Sera are serially diluted, and results are reported as endpoint titres, defined as the reciprocal of the last dilution of serum giving an optical density value above the predefined assay cut-off.

The following assay has been added:

Anti-Shigella conserved protein IgG ELISA

An anti-Shigella conserved protein (e.g., invasion plasmid antigen) total IgG ELISA will be used to profile natural exposure to Shigella.

Section 10.3.10.2 (Backup system in case the electronic reporting system does not work)

The following sentence has been modified:

If the electronic reporting system does not work, the investigator (or designee) must fax ***or email*** a completed, dated and signed paper Expedited Adverse Events Report to the study contact for reporting SAEs (refer to the Sponsor Information) or to the GSK GS department within 24 hours of becoming aware of the SAE.

Section 10.4.1.1.1 (Women not considered as women of childbearing potential)

The following bullet has been modified:

- Premenopausal female with ONE of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
  - ***Current bilateral tubal ligation or occlusion***

Section 11 (References)

The following reference has been added:

Cohen D, Ashkenazi S, Schneerson R, et al. Threshold protective levels of serum IgG to Shigella lipopolysaccharide: re-analysis of Shigella vaccine trials data. Clin Microbiol Infect. 2022;13:S1198-743X(22)00525-0

Detailed description of the Protocol Amendment 2:

Cover page:

In “Other study interventions”, the following study intervention has been modified:

- ~~Poliomyelitis,~~ d***D***iphtheria, tetanus, and pertussis vaccine (INFANRIX)

Protocol Sponsor Signatory Approval

In "Sponsor signatory", the following name has been changed:

~~Audino Pietro Mario Podda~~ ***Ashwani Kumar Arora***, GVGH Head of Clinical Development & Regulatory Affairs

Section 1.1 (Synopsis)

Under "Objectives endpoints", the following footnotes for Secondary Endpoints have been modified:

*This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~Clinical Study Report~~ ***Statistical Analysis Plan (SAP)***.

This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~ ***SAP*.

Under "Objectives endpoints", the following Tertiary Objective and Endpoints have been added:

• ***To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and 25% of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort***	• ***Antibody features (subclassing, affinity, avidity) and functional properties (induction of cellular phagocytosis and cellular cytotoxicity, natural killer [NK] cell activation, eosinophil and basophil degranulation, complement deposition, neutrophil activation/phagocytosis, dendritic cell phagocytosis, mucin binding, glycosylation, and Fc receptor alteration), as measured by systems serology analysis*** • ***Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous Shigella serotypes, as measured by SBA*** • ***Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine homologous and heterologous Shigella serotypes, as measured by opsonophagocytosis assay***

Under "Objectives and Endpoints", the following footnotes for Tertiary Endpoints have been modified:

This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~ ***SAP*.

This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~ ***SAP.

Section 1.2 (Schema)

In the top figure, the dotted vertical lines showing the interim analyses have been removed.

In the figure under "Stage 2 in Africa, Infants 9 months of age", the following text has been added under the dotted line between Day 113 and Day 253:

Interim analysis ***in dose-finding cohorts***

Section 1.3 (Schedule of Activities), Table 1 (Schedule of Activities for Stage 1)

Black filled bullets have been added on the row "Transcription of unsolicited AEs in the eCRF by the investigator or delegate" for Visit 2 (Day 8) and Visit 6 (Day 92 or Day 176) columns.

The following table footnote has been revised:

Note: The double-line borders following Day 29 indicate interim analyses in these participants, which will be performed on all data obtained up to Day 29. The double-line borders following Day 197 indicate ***interim or*** final analyses in these participants, which will be performed on all data obtained up to Day 197.

Section 1.3 (Schedule of Activities), Table 2 (Schedule of Activities for Adults 18 to 50 years of age in Stage 2)

Black filled bullets have been added on the row "Transcription of unsolicited AEs in the eCRF by the investigator or delegate" for Visit 2 (Day 8) and Visit 5 (Day 92) columns.

Section 1.3 (Schedule of Activities), Table 3 (Schedule of Activities for Children 24 to 59 months of age in Stage 2)

Black filled bullets have been added on the row "Transcription of unsolicited AEs in the eCRF by the investigator or delegate" for Visit 2 (Day 8) and Visit 5 (Day 92) columns.

Section 1.3 (Schedule of Activities), Table 4 (Schedule of Activities for Infants 9 months of age in Stage 2)

Black filled bullets have been added on the row "Transcription of unsolicited AEs in the eCRF by the investigator or delegate" for Visit 2 (Day 8), Visit 5 (Day 92), and Visit 8 (Day 260) columns.

The following footnote has been revised:

Note: The double-line borders following Day 113 indicate interim analyses in ~~these participants~~ ***the infant dose-finding cohort***, which will be performed on all data obtained up to Day 113 ***in the dose-finding cohort***. The double-line borders following Day 281 indicate ***interim analysis in the infant safety cohorts and final analysis***~~final analyses in these participants~~, which will be performed on all data obtained up to Day 281.

Section 1.3 (Schedule of Activities), Table 5 (Intervals between study visits)

An asterisk has been added to the title of the column "Allowed interval range" and the following corresponding footnote:

In case a participant is out of window for a particular Visit, the next Visit should be scheduled based on the originally planned protocol visit intervals and allowed interval ranges

Section 2.3.2 (Benefit assessment)

In the second paragraph, the following sentence has been added:

Vaccination with the investigational vaccine may provide protection against *S. sonnei* and *S. flexneri* bacteria. Vaccines administered as control and not routinely given will be provided free of charge and can provide protection against additional diseases (meningitis, typhoid, diphtheria, tetanus, pertussis). ***Additionally, children and infants who were not administered MENVEO as a control vaccine during the study will be offered the opportunity to receive a meningitis vaccine at the end of the study.***

Section 3 (Objectives and Endpoints), Table 6 (Study objectives and endpoints)

Under Secondary Endpoints, the following footnotes have been modified:

*This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~Clinical Study Report~~ ***Statistical Analysis Plan (SAP)***.

This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~*SAP*.

Under Tertiary Objectives and Endpoints, the following objective and endpoints have been added:

• ***To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and 25% of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.***	• ***Antibody features (subclassing, affinity, avidity) and functional properties (induction of cellular phagocytosis and cellular cytotoxicity, natural killer [NK] cell activation, eosinophil and basophil degranulation, complement deposition, neutrophil activation/phagocytosis, dendritic cell phagocytosis, mucin binding, glycosylation, and Fc receptor alteration), as measured by systems serology analysis.*** • ***Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous Shigella serotypes, as measured by SBA.*** • ***Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine homologous and heterologous Shigella serotypes, as measured by opsonophagocytosis assay.***

Under Tertiary Endpoints, the following footnotes have been modified:

This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~*SAP***.

This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~CSR~~SAP.***

Section 4.1 (Overall design)

In the third paragraph, the following phrase has been added:

For the study to proceed to Stage 2 in Africa, in addition to a favourable safety evaluation, ~~immunogenicity results need to fulfil Go/No Go criteria derived using~~ ***a comprehensive review of Stage 1 data will also be performed with the support of*** a Quantitative Decision Making (QDM) approach (refer to Section 9.2.1).

Section 4.1 (Overall design), Figure 1 (Study design overview)

In the top figure, the dotted vertical lines showing the interim analyses have been removed.

In the figure, under "Stage 2 in Africa, Infants 9 months of age", the following text has been added under the dotted line between Day 113 and Day 253:

Interim analysis ***in dose-finding cohorts***

Section 5.2.2 (Prior/Concomitant therapy)

The following exclusion criterion has been revised:

- A vaccine not foreseen* by the Study Protocol administered during the period starting at -21 days before the first dose (-28 days in the case of live vaccines) and ending after the last dose of study intervention administration**.

  *Vaccines allowed by the protocol include flu ***vaccines*** and COVID-19 vaccines in ~~adults~~ ***all participants,*** and EPI vaccines in children and infants. ***The recommended time intervals for administration of these vaccines is at least 7 days before or 7 days after (at least 14 days before or 14 days after in case of live vaccines) each dose of study intervention administration. In case these allowed vaccines are administered outside the aforementioned interval during the study, the participant should continue to receive the subsequent study intervention administration as per planned schedule.***

  In case emergency mass vaccination for an unforeseen public health threat (e.g., a pandemic) is ***recommended and/*organised by public health authorities outside the routine immunisation programme, the time period described above can be reduced if, necessary for that vaccine, provided it is ~~licensed~~ used according to ~~its Product Information.~~***the local governmental recommendations and that the sponsor is notified accordingly. When regulations allow, the recommended time intervals for administration of these vaccines is at least 7 days before or 7 days after (at least 14 days before or 14 days after in case of live vaccines) each dose of study intervention administration.***

Section 5.4 (Screen failures)

The following sentence has been modified:

A screen failure is an individual who consents to participate in this this study but is not entered in the study~~/randomised to a study intervention~~***(was not randomised, did not receive study intervention, and did not undergo a post-screening study procedure)***.

Section 5.5 (Criteria for temporarily delaying enrolment and study intervention administration)

The following text has been modified and added as a bullet:

- Participants with a positive COVID-19 test ~~in the 28 days before a planned vaccination will be allowed to be vaccinated only starting from 28 days after the date of their first positive COVID-19 test~~ ***before a planned study intervention administration will be allowed to receive the study intervention only if at least 14 days have passed since the first positive test***.

Section 6.1 (Study interventions administered)

In the second paragraph, the following sentence has been modified:

The candidate vaccine, altSonflex1-2-3, will be administered intramuscularly by unblinded study site staff and contains 15 µg ***(Dose C), 7.5 µg (Dose B), or 3.75 µg (Dose A)*** of OAg for each antigen component ***(refer to the Study Procedures Manual [SPM] for additional details)***.

Section 6.3.5 (Allocation of participants to assay subsets)

The following bullets have been modified:

- Stage 1 Adults Subset: 25% of participants will be randomly selected from each adult treatment study group in Stage 1 (ST1_Adults_Dose C_GR1", "ST1_Adults_Dose C_GR2", "ST1_Adults_Placebo_GR1", and "ST1_Adults_Placebo_GR2"). For this randomly selected subset of participants, ~~all~~ ***available*** samples taken at Days 1, 15, 29, 85/169 and 113/197 will be tested ~~with SBA for bactericidal antibodies against *S. Sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a.~~ ***to fulfil tertiary objectives (SBA, systems serology analysis, and opsonophagocytosis assay).***

- Stage 2 Infants Dose-Finding Cohort Subset: 25% of participants will be randomly selected from each infant study intervention study group of the dose-finding cohort ("ST2_Infants_Control_Dose find", "ST2_Infants_Dose A_Dose find", "ST2_Infants_Dose B_Dose find", and "ST2_Infants_Dose C_Dose find"). For this randomly selected subset of participants, all ***available***-samples taken at Days 1, 29, 85, 113, 253, and 281 will be tested ~~with SBA for bactericidal antibodies against *S. Sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a.~~ ***to fulfil tertiary objectives (SBA, systems serology analysis, and opsonophagocytosis assay).***

Section 6.3.6 (Blinding and unblinding)

In the second paragraph, the following sentence has been modified:

~~As per study design, I~~***I***n Stage 1, investigators will be~~come~~ unblinded to the vaccination schedule.~~ From Visit 5 onwards.~~

Section 6.3.6.2 (Unblinding prior to regulatory reporting of SAEs)

The following paragraphs have been modified:

GSK policy requires unblinding of any unexpected SAE which is attributable/suspected to be attributable to the study interventions, prior to regulatory reporting. ~~Vaccines Clinical Safety and Pharmacovigilance (VCSP)~~ ***GSK Global Safety (GS)*** is responsible for unblinding the study intervention assignment within the timeframes defined for expedited reporting of SAEs (refer to the Section 10.3.10.1).

In addition, GSK ~~VCSP~~ ***GS*** staff may unblind the intervention assignment for any participant with a Suspected Unexpected Serious Adverse Reaction (SUSAR) or a SAE that is fatal or life-threatening. For SAEs requiring expedited reporting to 1 or more regulatory agencies, a copy of the report containing participant's intervention assignment may be sent to investigators in accordance with local regulations and/or GSK policy.

Section 6.6 (Continued access to study intervention after the end of the study)

The following sentence has been modified and an additional sentence has been added:

It is the responsibility of the investigator ***(supported by GVGH)*** to determine the medical care of the non-responder as per local/regional practices, such as ~~re-vaccination~~ ***revaccination against measles and rubella in Stage 2***.

After unblinding, the investigator will also be supported to offer vaccination with a single dose of a locally licensed 4-component meningitis vaccine to all children and infants who had not received MENVEO during the study.

Section 8 (Study Assessments and Procedures)

The following bullet has been modified:

- If despite best efforts, it is not possible to ~~collect the biological samples~~ ***perform the Visits*** within the interval predefined ~~(see Table 5)~~***in Table 5 of*** the protocol, then the interval may be extended for the specified Visits up to a maximum length of days as presented in Table 13.

Section 8 (Study Assessments and Procedures), Table 13 (Intervals between study visits during special circumstances)

Rows and an asterisk to the column "Allowed interval range" with corresponding footnote have been added to Table 13:

Interval	Planned visit interval	Allowed interval range*
Stage 1 – Adults 18 to 50 years of age – Groups 1 and 2		
Visit 1→Visit 4	28 days	28 days-38 days
Stage 1 – Adults 18 to 50 years of age – Group 1		
Visit 4→Visit 5	***56 days***	***50 days-64 days***
Visit 5→Visit 7	28 days	28 days-38 days
Stage 1 – Adults 18 to 50 years of age – Group 2		
Visit 4→Visit 5	***140 days***	***130 days-150 days***
Visit 5→Visit 7	28 days	28 days-38 days
Stage 2 – Adults 18 to 50 years of age		
Visit 1→Visit 3	28 days	28 days-38 days
Visit 3→Visit 4	***56 days***	***50 days-64 days***
Visit 4→Visit 6	28 days	28 days-38 days
Stage 2 – Children 24 to 59 months of age		
Visit 1→Visit 3	28 days	28 days-38 days
Visit 3→Visit 4	***56 days***	***50 days-64 days***
Visit 4→Visit 6	28 days	28 days-38 days
Stage 2 – Infants 9 months of age		
Visit 1→Visit 3	28 days	28 days-38 days for dose-finding cohort
Visit 3→Visit 4	***56 days***	***50 days-64 days***
Visit 4→Visit 6	28 days	28 days-38 days for dose-finding cohort
Visit 6→Visit 7	***140 days***	***130 days-150 days***
Visit 7→Visit 9	28 days	28 days-38 days

In case a participant is out of window for a particular visit, the next Visit should be scheduled based on the originally planned protocol visit intervals and allowed interval ranges

Section 8.1.1 (Biological samples), Table 15 (Laboratory assays)

The following paragraph has been modified:

Up to 150 mL of blood will be collected 28 days after the second study intervention administration in Stage 1 from ~~the first~~ 20 participants who gave specific informed consent for the purpose of extraction of peripheral blood mononuclear cells (PBMC) and sera***, and considered eligible by the site staff (refer to the SPM for further details).*** Extracted materials will be used to generate monoclonal antibodies, which may play a role in protection against *Shigella* infection. This blood collection is not linked to any endpoint in this study but related to separate exploratory research on monoclonal antibodies against *Shigella*.

Section 8.1.2 (Laboratory assays)

The following sentence has been added:

Exploratory systems serology analysis assays, panel SBA, and the opsonophagocytosis assays will be performed by the GVGH laboratory or a laboratory designated by GVGH.

Section 8.2.2.1 (Staggered vaccination), Table 17 (Safety evaluation schedule)

In Table 17, the last row of the table has been modified:

Safety evaluation	Safety data reviewed*	Vaccination approval to
Stage 1: Adults 18 to 50 years of age		
#1: Blinded**	Up to 7 days after 1st vaccination of first 12 participants	Administration of 2nd vaccination to first 12 participants + administration of 1st vaccination to subsequent 90 participants in Stage 1
#2: Unblinded	Up to 28 days after 1st vaccination of all 102 participants	Administration of 1st vaccination to adults in Stage 2
Stage 2: Adults 18 to 50 years of age		
#3: Unblinded	Up to 7 days after 1st vaccination in all adults in Stage 2	Administration of 1st vaccination with Dose B to children
Stage 2: Children 24 to 59 months of age		
#4: Blinded**	Up to 7 days after 1st vaccination in all children with Dose B	Administration of 1st vaccination with Dose C to children, Administration of 2nd vaccination with Dose B to children
#5: Unblinded	Up to 28 days after 1st vaccination in all children with Dose C	Administration of 1st vaccination with Dose A to infants
Stage 2: Infants 9 months of age		
#6: Blinded**	Up to 7 days after 1st vaccination in all infants with dose A in the safety cohort	Administration of 1st vaccination with Dose B to infants in the safety cohort, Administration of subsequent vaccinations with Dose A to infants in the safety cohort
#7: Blinded**	Up to 7 days after 1st vaccination in all infants with Dose B in the safety cohort	Administration of 1st vaccination with Dose C to infants in the safety cohort, Administration of subsequent vaccinations with Dose B to infants in the safety cohort
#8: Unblinded	Up to 28 days after 1st vaccination in all infants with Dose C in the safety cohort	Administration of 1st vaccination to infants in the dose-finding cohort, Administration of subsequent vaccinations to infants of Dose C in the safety cohort
#9: Unblinded	Up to 7 days after ***the*** 1st vaccination ***in*** ~~all infants of~~ ***the dose-finding cohort of all infants who were vaccinated in the first 7 weeks since the 1st vaccination in the dose-finding cohort***	Administration of ~~2nd~~ ***subsequent*** vaccination***s*** to infants in the dose-finding cohort

Section 8.2.2.1.2 (Stage 2 [Africa])

The last paragraph has been modified:

~~When all infants~~ ***Seven weeks after the first infant*** in the dose-finding cohort ~~have~~ ***received the first vaccination, all available safety data*** ~~from (e.g.~~ ***i.e,. data collected up to***-the 7 days after the first vaccination of infants in the dose-finding cohort and all available safety data ~~28 days after second vaccination with Dose C in~~ ***from***-the safety cohort of adults and children will be reviewed in an unblinded manner to decide if the study can continue with the second vaccination of infants in the dose-finding cohort (Table 17, safety evaluation #9). ***This approach is to avoid having infants who receive the first vaccination in the dose-finding cohort being out of window for the second vaccination, in consideration for recruitment and operational timelines.***

Section 8.2.2.2 (Outcome of safety evaluation)

The third bullet has been modified:

- If a safety signal is observed during blinded or unblinded safety data reviews or if any of the holding rules 2a-~~c~~***b*** (refer to Table 18) is met, the iSRC Chair (or his/her representative) is responsible for the urgent communication to the GVGH PP, including any rationale for a decision to put the vaccination on hold or not.

Section 8.2.2.3 (Study holding rules)

In the first 2 paragraphs, the following words have been modified:

The safety holding rules are defined in Table 18. Holding rules 1a-d will be assessed by the investigator on a continuous basis irrespective of the number of participants enrolled and meeting any of these holding rules will trigger a hold of vaccination irrespective of number of participants enrolled and/or timing of the event relative to vaccination. Holding rules 2a-~~c~~***b*** will be assessed by the iSRC during the safety evaluations on unblinded data. The evaluation for the percentage of participants needed to trigger a hold will be based on the ~~planned~~ number of participants receiving altSonflex1-2-3 at the time of the safety evaluation.

These holding rules have been written under the assumption that the safety data from all participants will be available. If the data from ~~all~~ ***some of the*** participants are not available (i.e., in case a participant is lost to follow-up), then the holding rules will be assessed on a pro-rata basis.

Section 8.2.2.3 (Study holding rules), Table 18 (Study holding rules)

The last 2 rows of Table 18 have been modified and a footnote has been added:

Holding rule	Event	Number or percentage of participants needed to trigger a hold	
		Safety cohorts	Dose-finding cohorts
1a	Death or any life-threatening SAE regardless of causality	≥1	
1b	Any non-life-threatening SAE that can be reasonably attributed to the vaccination	≥1	
1c	Any withdrawal from the study (by investigator or participant request) following a Grade 3 AE that can be reasonably attributed to the vaccination	≥1	
1d	Fever >40°C that can be reasonably attributed to the vaccination OR Any administration-site or systemic solicited event leading to hospitalisation OR Necrosis at the injection site, each within the 7-day (days 1-7) post-vaccination period	≥1	
2a	Any Grade 3 solicited administration-site event (lasting 48h or more) in an investigational group*, within the 7-days (Days 1-7) post-vaccination period	***10%***	***10%*****

2b	Any Grade 3 unsolicited AE in an investigational group*, that can be reasonably attributed to the vaccination, within the 7-day (day 1-7) post-vaccination period or Any Grade 3 abnormality in prespecified haematological or biochemical laboratory parameters** in an investigational group* within the 7-day (day 1-7) post-vaccination period	***≥1 in all safety cohorts, except for ≥3 for Dose 2 data evaluation in Stage 1***	***10%******

AE=adverse event; SAE=serious adverse event
*Investigational group refers to ~~groups~~***an individual study** group receiving the altSonflex1-2-3 vaccine
~~**Please refer to Section 10.8 for the toxicity grading scales of laboratory parameters. Laboratory parameters that are not included in the toxicity grading scales will not be graded.~~
In-stream blinded monitoring will evaluate the total number of participants vaccinated at the time of the safety evaluation (candidate vaccine and control recipients) and will trigger an ad hoc iSRC if the percentage threshold is reached. The iSRC will confirm if the holding rule has been met based on the number of participants who have received altSonflex1-2-3 vaccine in the specific investigational group.
***Please refer to Section 10.8 for the toxicity grading scales of laboratory parameters. Laboratory parameters that are not included in the toxicity grading scales will not be graded.

Section 8.2.2.3 (Study holding rules)

The last bullet of the Section has been modified:

- The GSK-GVGH clinical team in collaboration with the GSK ***GS*** ~~Safety~~ Department will further evaluate the case with the iSRC and GSK ~~Vaccines Safety Monitoring Board~~***Global Safety Board*** and will take the decision to stop or to restart the vaccination. All site staff will be informed about that final decision by the GVGH SDL and GVGH PP.

Section 8.3.2.1 (Clinically significant abnormal laboratory findings)

The following bullet has been added:

- ***In case abnormal values are not considered clinically significant by the investigator, additional evaluations could be requested by the GVGH project physician.***

Section 8.5 (Genetics and/or Pharmacogenomics)

The following paragraph has been modified:

To minimise any risk of autoimmune reactions, blood samples collected for safety during Screening will undergo sampling for the HLA-b27 genetic marker. Participants with a positive test result will ***not*** be ~~excluded~~ ***included*** ~~from~~ ***in*** the study. ***In case of participant rescreening, this test should not be repeated***.

Section 9.2.1 (Stage 1 Quantitative Decision Making)

The following paragraphs has been modified:

A QDM approach will be used to ~~drive~~***support*** the Go/No-Go decision to proceed to Stage 2. The endpoint measuring number of adults in Europe 18 to 50 years of age in Stage 1 achieving a GVGH ELISA level equivalent to 1:1600 titre against *S. sonnei* LPS at Day 29 was selected as the most clinically relevant for decision making. A Minimum Value (MV) of 60% and a Target Value (TV) of 70% for participants in the vaccine group with GVGH ELISA level below 1:1600 titre against *S. sonnei* LPS at baseline (Day 1) achieving such a threshold at Day 29 have been defined for the clinical development programme. MV and TV are the critical values that are the basis for defining the threshold limits (vertical solid lines in Figure 2) that will determine the Go/No-Go decision in the context of the QDM approach.

Approximately 102 participants will be randomised to achieve 61 evaluable participants in the vaccine arm of Stage 1**.** Assuming a 10% drop-out rate, including removal of participants with GVGH ELISA level ≥1:1600 titres at baseline (Day 1), in Stage 1 and therefore 61 evaluable participants in the participants receiving altSonflex1-2-3, and defining a MV of 60% and TV of 70% of participants achieving an equivalent 1:1600 ELISA level against *S. sonnei* titres, an observed percentage of participants ≥67.2% achieving the equivalent 1:1600 titre threshold ensures with a more than 80% probability that the true percentage of participants is greater than or equal to 60% (MV). This leads to a Go decision (green zone in Figure 2). ~~Conversely,~~***A***n observed percentage of participants ~~≤ 60.7% ensures more than 90% probability that the true percentage of participants is less than or equal to 70% (TV). This leads to a No-Go decision (red zone in Figure 2). Observed values between 60.7% and 67.2% will fall in the "consider zone" (yellow zone in Figure 2). In that case, other factors will be considered as well~~ ***≤67.2% (yellow and red zones) will trigger a comprehensive analysis of all available immunogenicity data across serotypes and/or after the second vaccination in Stage 1*** to make the Go/No-Go decision.

Section 9.3 (Analysis sets), Table 26 (Analysis sets)

The following row of Table 26 has been modified:

Analysis set	Description
Enrolled	~~Participants who were randomised or received a study intervention or had blood drawn during screening. The allocation in a group is done in function of the administered treatment~~ ***All participants who entered the study (who were randomised or received study intervention or underwent a post-screening study procedure).*** ***Note: participants who never passed screening (screening failures) and participants screened but who never enrolled into the study (met eligibility criteria but were not needed) are excluded from the Enrolled analysis set***

Section 9.3.1 (Criteria for elimination from analysis)

The following criterion has been modified and split into 2 criteria

- Use of any vaccine not foreseen by the Study Protocol administered during the entire period of participant's study participation ***(refer to Section 5.2.2)***~~, with the exception of flu vaccines, COVID-19 vaccines, and vaccines administered as part of EPI.~~
- ***Use of foreseen vaccines outside the recommended time window (refer to Section 5.2.2)***.

The following bullet has been modified:

- Unblinding after any study intervention has been ~~performed~~ ***administered***.

The following sentence has been added:

Other criteria for elimination from analyses will be reported in the SAP.

Section 9.4.1 (Primary endpoints)

The following sentence has been modified:

Safety analysis will be based on the ***Exposed,*** Solicited Safety and Unsolicited Safety sets.

Section 9.4.3 (Tertiary endpoints)

The sentences in the column "Statistical Analysis Methods" have been modified:

For all the above, within-participant GMRs will be computed for GMT with 95% CI at each time point after vaccination versus baseline (Day 1). ~~Exploratory immunology:~~

- ~~If available and deemed appropriate, any further exploratory immunology may be reported by group and time point using descriptive summary statistics. This will be described in a SAP finalised before database unblinding/freezing~~ ***Additional exploratory immunology including system serology analysis, panel SBA, and opsonophagocytosis assay, will be performed on the aforementioned subsets of 25% randomly chosen participants and will be reported by group and timepoint using descriptive summary statistics. This analysis will be described in a dedicated SAP.***

Section 9.5 Interim analyses

The following sentence has been modified:

To accelerate evaluation of vaccine immunogenicity in the target population and facilitate planning of future studies, ~~2~~***3*** study interim analyses and 1 final analysis (Stage 1 and Stage 2) will be performed as described in Section 9.5.1.s

Section 9.5.1 (Sequence of analyses)

The Section has been modified:

All analyses before unblinding will be conducted on ***cumulative*** data as clean as possible.

The sequence of interim and final analyses will be the following:

a. Stage 1 interim analysis at Day 29 (28 days after first vaccination) on immunogenicity ~~in participants receiving altSonflex1-2-3. This analysis will be performed using the QDM approach by GSK unblinded statistician external to the project and Statistical Analysts to generate a Go/No-Go decision.~~***in all Stage 1 participants. This analysis will support a QDM approach, which will be part of a GO/NO-GO decision for the study to proceed to Stage 2; to be carried out by the IDAC*** No individual listings or data with the participants identifying information will be disseminated.

b. Stage 1 interim analysis at Day 197 on immunogenicity and safety data on all Stage 1 participants in order to obtain early immunogenicity and safety data from Stage 1; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

~~c. Stage 2 interim analysis at Day 281 in infant safety cohort: in order to obtain early immunogenicity data analysis will be performed when all immunogenicity data in infant safety cohort will available; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.~~

c. Stage 2 interim analysis at Day 113 ~~(28 days after second vaccination) in infant dose-finding cohort:~~ ***on immunogenicity data from infant dose-finding cohort (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 cohorts,*** in order to obtain early immunogenicity data analysis ~~will be performed on immunogenicity from the dose-finding cohort~~; to be carried out by IDAC. No individual listings or data with the participants identifying information will be disseminated.

d. ~~Stage 1 final analysis and Stage 2 final analysis at Day 197 on immunogenicity and safety data on all Stage 1 participants and at Day 281 (28 days after last vaccination in infants) on~~ ***Final analysis including Stage 1 final analysis at Day 197 and Stage 2 final analysis at Day 281 on all*** immunogenicity and safety data once Stage 2 of the trial is concluded; to be carried out by the GVGH Project Statistician and GSK Statistical Analysts.

In case of a No-Go decision ~~in the first interim analysis (a)~~***after the first (a) or second (b) interim analyses***, a final analysis will be performed at Day 197 on immunogenicity and safety data ***of*~~on~~ all Stage 1 participants, to be carried out by GVGH Project Statistician and GSK Statistical Analysts.

The ~~final~~ Clinical Study Report (CSR) will contain at least the final analyses of all primary and secondary endpoints (in case of a Go decision ~~in interim analysis "a"~~[22]). Only Stage 1 endpoints will be part of the ~~final~~ CSR in case of a No-Go decision (interim analysis "a" ***and "b"***).

~~If d~~***D***ata for tertiary endpoints ***that*** become available at ~~a~~ later timepoint***s~~, these~~ will be documented in annex(es) to the final CSR or in an integrated final CSR.

Section 10.1.3 (Informed consent process)

The following paragraph has been modified:

~~If a participant is rescreened outside the screening window, a new ICF must be signed by the participant/participant's parent(s)/LAR(s).~~

For participants who are rescreened, participants/participants' parent(s)/LAR(s) are required to sign a new ICF, only if there are changes to the original ICF. If there are no changes to the original ICF, participants/participants' parent(s)/LAR(s) should confirm that they still agree to be part of the study. This information should be captured in the participant source document.

Section 10.1.6 (Dissemination of clinical study data)

The first sentence of the Section has been modified:

The key design elements of this protocol and results summaries will be posted on www.ClinicalTrials.gov, ***https://pactr.samrc.ac.za,*** and/or GSK Clinical Study Register in compliance with applicable regulations/GSK policy.

Section 10.2 (Appendix 2: Clinical laboratory tests)

Sentences have been modified and additional text added:

Serum bactericidal activity in serum:

Serum bactericidal activity against *S. sonnei* ~~and *S. flexneri* 1b, 2a and 3a~~ ***S. flexneri 1b, S. flexneri 2a, S. flexneri 3a, and other heterologous strains,*** will be measured by luminescence-based SBA, based on a method previously reported [Necchi, 2017; Rossi, 2020]. Results are expressed in serum titres, defined as serum dilutions giving 50% inhibition of bacterial growth (IC_{50}).

Opsonophagocytosis assay

Opsonophagocytic killing activity (OPK) against a panel of Shigella strains will be measured by a traditional colony factor unit-based method using HL60 or Thp-1 cells. Results are expressed in OPK titres, defined as serum dilutions giving 50% inhibition of bacterial growth (IC_{50}).

Measles and rubella ELISA:

Anti-measles and anti-rubella IgG antibodies will be determined by ELISA using commercial kits.

System serology assay panel:

A panel of assays will be used to conduct a system serology analysis, based on a previously reported method [Chung 2015], to evaluate the following antibody properties and functional activities: antibodies subclassing, antibody affinity, antibody avidity, antibody dependent cellular phagocytosis, antibody dependent cellular cytotoxicity, antibody dependent NK cell activation, antibody dependent eosinophil degranulation, antibody dependent basophil degranulation, antibody dependent complement deposition, antibody dependent neutrophil activation/phagocytosis, antibody dependent dendritic cell phagocytosis, antibody dependent mucin binding, Fc receptor array, and antibody glycosylation.

Section 10.2.1 (Protocol required safety laboratory assessments), Table 27 (Protocol required safety laboratory assessments)

In Table 27, the following clinical chemistry parameter applicable to "All participants" has been modified:

All participants	**Clinical chemistry**	• Blood Urea ~~Nitrogen~~

Section 10.3.8.3 (Updating of SAE and pregnancy information after removal of write access to the participant's eCRF

The following sentence has been modified:

The updated report should be faxed to the Study contact for reporting SAEs (refer to the Section 8.3.3.1) or to ***the*** GSK ~~VCSP~~ ***GS*** department within the defined reporting time frames specified in the Table 23.

Section 10.3.10.2 (Backup system in case the electronic reporting system does not work)

The following sentence has been modified:

If the electronic reporting system does not work, the investigator (or designee) must fax a completed, dated and signed paper Expedited Adverse Events Report to the study contact for reporting SAEs (refer to the Sponsor Information) or to ***the*** GSK ~~VCSP~~ ***GS*** department within 24 hours of becoming aware of the SAE.

Section 10.5 (Appendix 5: Genetics)

The following sentence has been added:

In case of participant rescreening, this test should not be repeated since the result is not expected to change in the participant lifetime.

Section 10.10.1 (List of abbreviations)

The following abbreviation has been modified:

GS: ***Global Safety (GSK)***

OPK: ***opsonophagocytic killing activity***

~~VCSP Vaccines Clinical Safety and Pharmacovigilance (GSK)~~

Section 11 (References)

The following reference has been added:

Chung AW, Kumar MP, Arnold KB, et al. Dissecting polyclonal vaccine-induced humoral immunity against HIM using Systems Serology. Cell. 2015;163: 988-98.

Detailed description of Protocol Amendment 1

Section 1.1 (Synopsis):

Under "Objectives and endpoints", the following primary endpoint has been modified:

- Anti-serotype specific *Shigella* lipopolysaccharide (LPS)* serum immunoglobulin G (IgG) titres as measured by reference enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the ~~booster~~ ***third*** vaccination (Day 281).

Under "Objectives and endpoints", the following secondary endpoint has been modified:

- Anti-serotype specific ***Shigella*** LPS* serum IgG GMTs, in infants 9 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).

Under "Objectives and endpoints", the following secondary endpoint asterisks have been modified:

*This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~Statistical Analysis Plan~~ ***Clinical Study Report (CSR)***.
This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~*CSR*.

Under "Objectives and endpoints", the following tertiary endpoint asterisks have been modified:

This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~ ***CSR*.
This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~CSR*.

Section 1.2 (Schema) and Section 4.1 (Overall design), Figure 1 (Study design overview)

The box related to the Screening period in the figure for Stage 1 in Europe (Adults 18 to 50 years of age) has been modified:

Day ~~-56~~-***28*** to -1

Section 1.3 (Schedule of Activities)

In Table 1 (Schedule of Activities for Stage 1), the cell related to the Screening period on the following row related to Timepoints has been modified:

Timepoints	Day -~~57~~*28* to Day -1	Day 1	Day 4	Day 8	Day 15	Day 29	Day 85	Day 169	Day 88	Day 172	Day 92	Day 176	Day 113	Day 197

In Table 4 (Schedule of Activities for Infants 9 months of age in Stage 2), the following footnote has been modified:

[4]The ~~booster~~ ***control*** vaccination will consist of the INFANRIX vaccine

Section 3 (Objectives and endpoints)

In Table 6 (Study objectives and endpoints), the following primary endpoint has been modified:

- Anti-serotype specific *Shigella* lipopolysaccharide (LPS)* serum immunoglobulin G (IgG) titres as measured by reference enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the ~~booster~~ ***third*** vaccination (Day 281).

In Table 6 (Study objectives and endpoints), the following secondary endpoint has been modified:

- Anti-serotype specific ***Shigella*** LPS* serum IgG GMTs, in infants 9 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281).
- Anti-rubella IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1~~ and Day 253~~) and at 28 days after ~~each~~ ***the*** second MR-VAC vaccination (Day 281).

In Table 6 (Study objectives and endpoints), the following secondary endpoint asterisks have been modified:

*This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~Statistical Analysis Plan~~ ***Clinical Study Report (CSR)***.

This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~*CSR***.

In Table 6 (Study objectives and endpoints), the following tertiary endpoint asterisks have been modified:

This threshold (1:800, known as "Cohen's Threshold") has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017] and will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~ ***CSR*.
This threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the ~~SAP~~CSR***.

Section 4.2.1 (Rationale for choice of vaccination route and schedule)

The following bullet has been added:

- ***Infants are likely to require more vaccinations to achieve adequate immune responses compared to older children and adults. A previous Shigella vaccine glycoconjugate failed to protect children when 2 vaccinations were administered [Passwell 2010]. Additionally, other Shigella vaccines in development are using at least 3 vaccinations in infants (NCT04602975 and NCT04056117).***

Section 4.2.2 (Rationale for choice of study population)

In the second paragraph, the following sentence has been added:

[…] The vaccine will first be tested in adults in Europe, a population not endemic for shigellosis and a setting considered as low-risk and low vulnerability, with high access to healthcare. ***The age range 18 to 50 years of age chosen for adults is consistent with previous Shigella studies performed by GVGH and also other studies investigating Shigella vaccines.*** Upon demonstrating positive safety and immunogenicity outcomes, the candidate vaccine will be tested in a population endemic for shigellosis, in adults in Africa. […]

Section 4.2.3 (Rationale for choice of control vaccines)

At the end of this section, an additional sentence has been added:

[…]

For the infants in Africa, MENVEO and INFANRIX will be administered because the timing of administration matches their recommended administrations.

Active comparator vaccines are being used in Stage 2 in Africa to provide some benefit to study participants randomised to the control groups of the study. The vaccines selected would not be routinely available to the population in the study geographical region outside the clinical study setting.

Section 4.3 (Justification for dose)

In the second bullet, the following sentences have been added:

- […] The starting dose of OAg (15 µg) in adults is selected to be higher than the dose used in the previous CHIM study (NCT03527173), which showed negative results for efficacy with 1.5/25 µg OAg/protein and insufficient immunogenicity. ***An O-antigen dose of 25 µg as a glycoconjugate vaccine showed high immunogenicity and 74% protective efficacy against shigellosis in adults [Cohen, 1997]. Also, a synthetic Shigella conjugate investigational vaccine, SF2a-TT15, containing 10 mg of O-antigen induced a much stronger immune response than one containing 2 mg [Cohen 2021], supporting the rationale that an O-antigen content much higher than 1.5 mg is needed for the GMMA vaccine as well.***

In the fifth bullet, the following sentence has been modified:

- Previous toxicology studies with 1790GMMA and Sonflex-1-2-3 showed that doses used were well tolerated and did not show evidence of systemic toxicity. Results from a toxicology study with ***the highest dose of*** altSonflex1-2-3 ~~is ongoing and is expected to~~ ***have*** show***n*** a similar ***safety*** profile~~ based on the lower GMMA protein and Lipid A contents~~.

Section 5.1 (Inclusion criteria)

The following inclusion criterion has been modified:

- Healthy participants as established by medical history***,*** ~~and~~ clinical examination, ***and laboratory assessment*** ~~before entering the study~~.

Section 5.2 (Exclusion criteria)

The following sentence has been modified:

The following criteria should be checked at the time of study entry. The potential participant ~~MAY~~ ***MUST*** NOT be included in the study if ANY exclusion criterion applies:

Section 5.2.1 (Medical conditions)

The following exclusion criteria have been modified:

All participants:

- Known exposure to *Shigella* during lifetime of the participant as confirmed during interview with the participant or documented by patient records (e.g., history of microbiologically-confirmed *Shigella* infection), ***recent travel* (within 2 years) to a country where Shigella or other enteric infections are endemic, or recent occupation* (within 3 years) involving Shigella species***

Exclusion due to travel or occupation is applicable only to Adults 18 to 50 years of age in Europe (Stage 1). Refer to Section 8.2.1 for additional instructions and Section 10.9 for the list of countries considered highly endemic for Shigella.

- Progressive, unstable, or uncontrolled clinical conditions.
- History ***(known or suspected)*** of any reaction or hypersensitivity likely to be exacerbated by any component of the study ***vaccine.***
- […]

Section 5.2.2 (Prior/Concomitant therapy)

Under the heading "All participants", the following exclusion criterion has been modified:

- A vaccine not foreseen***** by the Study Protocol administered during the period starting at -21 days before the first dose (-28 days in the case of live vaccines) and ending after the last dose of study intervention administration****,**

  ****Vaccines allowed by the protocol include flu and COVID-19 vaccines in adults and EPI vaccines in children and infants.***

  ******In case emergency mass vaccination for an unforeseen public health threat (e.g., a pandemic) is organised by public health authorities outside the routine immunisation programme, the time period described above can be reduced if, necessary for that vaccine, provided it is licensed and used according to its Product Information.

Under the heading "Adults 18 to 50 years of age", the following exclusion criterion has been modified:

- Administration of immunoglobulins and/or any blood products or plasma derivatives***, or bone marrow transplantation,*** during the period starting 3 months before the first dose of study vaccine or planned administration during the study period.

Under the heading "Children 24 to 59 months of age", the following exclusion criterion has been modified:

- Administration of immunoglobulins and/or any blood products or plasma derivatives***, or bone marrow transplantation,*** during the period starting 3 months before the first dose of study vaccine or planned administration during the study period.

Under the heading "Infants 9 months of age", the following exclusion criterion has been modified:

- Administration of immunoglobulins and/or any blood products or plasma derivatives***, or bone marrow transplantation,*** from birth or planned administration during the study period.

Section 7.1.1 (Contraindications to subsequent study intervention(s) administration

The following contraindication criterion bullet has been added:

- ***Participants who experienced any immediate allergic reaction, including anaphylaxis, within 24 hours after any previous study vaccination.***

Section 8.1.1 (Biological samples)

The following paragraph has been added:

Up to 150 mL of blood will be collected 28 days after the second study intervention administration in stage 1 from the first 20 participants who gave specific informed consent for the purpose of extraction of peripheral blood mononuclear cells (PBMC) and sera. Extracted materials will be used to generate monoclonal antibodies, which may play a role in protection against shigella infection. This blood collection is not linked to any endpoint in this study but related to separate exploratory research on monoclonal antibodies against Shigella.

Section 8.2.1.2 (Medical/vaccination history)

Under the heading "All participants", the following text has been added:

As part of the participant/parent(s)/LAR(s) interview during Screening and to evaluate whether the participant meets exclusion criterion of known exposure to Shigella, the investigator or delegate should assess whether there are any medical records showing confirmed microbiological diagnosis of Shigella diarrhoea in the lifetime of the participant. Additionally, any medical history suggestive of Shigella diarrhoea should be obtained based on the following features and the medical judgement of the Investigator:

- ***History of watery diarrheoa with or without blood associated with crampy abdominal pain***
- ***History of fever, toxicity and anorexia***
- ***Features suggestive of Haemorrhagic Uraemic Syndrome***
- ***Features of acute abdomen following diarrhoeal illness***
- ***History of similar illness in other household members***

The following heading and paragraph have been added:

Adults 18 to 50 years of age in Europe:

As part of the participant/LAR(s) interview during Screening and to evaluate whether the participant meets exclusion criterion of known exposure to Shigella, the investigator or delegate should obtain participant travel history (within 2 years) to countries highly endemic for Shigella (refer to Section 10.9 for the list of countries) or occupational exposure (within 3 years) to Shigella. Examples of occupational exposure

could include caring for non-human primates, working in child day-care facilities, and male commercial sex workers who have sex with men.

Section 8.2.2.1.2 (Stage 2 [Africa])

Under the heading "Infants 9 months of age", the following words have been modified:

After approval, 20 infants will receive 3 ***primary*** vaccinations ~~(2 primary vaccinations and 1 booster vaccination)~~ of either Dose A of altSonflex1-2-3 or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the ~~booster~~***third*** vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and INFANRIX for the ~~booster~~***third*** vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will be vaccinated in parallel.

[…]

After approval, 20 infants will receive 3 ***primary*** vaccinations ~~(2 primary vaccinations and 1 booster vaccination)~~with either Dose B or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the ~~booster~~***third*** vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and INFANRIX for the ~~booster~~***third*** vaccination. The first 5 infants will the receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will be vaccinated in parallel.

[…]

After approval, 20 infants will receive 3 primary vaccinations ~~(2 primary vaccinations and 1 booster vaccination)~~ with either Dose C or a control vaccine (1:1 randomisation ratio). They will also receive an EPI vaccination with MR-VAC at 28 days after first vaccination and at 28 days after the ~~booster~~***third*** vaccination. The control vaccines will be MENVEO for the first and second vaccinations, and INFANRIX for the ~~booster~~***third*** vaccination. The first 5 infants will then receive the first vaccination in a sequential manner with a time interval of at least 60 minutes between participants. The subsequent 15 infants will be vaccinated in parallel.

[…]

After approval, a total of 328 infants will be vaccinated with either Dose A, Dose B, or Dose C of the altSonflex1-2-3 candidate vaccine, or with a control vaccine (1:1:1:1 randomisation ratio). The vaccination schedule will be the same as in the infant safety cohort. However, in this cohort, the EPI vaccination with MR-VAC will be given concomitantly with the first vaccination and with the ~~booster~~***third*** vaccination.

[…]

Section 8.3.1 (Time period and frequency for collecting AE, SAE, and other safety information)

In Table 19 (Timeframes for collecting and reporting of safety information in Adults in Stage 1), the first row has been modified:

Event	SCR-D-~~28~~ ~~56~~ to D-1*	VAC 1 D1	D8	D29	VAC 2 D85/D169	D92/D176	D113/D197

Section 9.2.2 (Stage 2 Dose-finding cohort)

The following word has been modified:

In the absence of previous studies on the altSonflex1-2-3 formulation, the minimum increment to define the preferred dose has been chosen by the GVGH clinical team to be a 2-fold increase between groups 28 days after the ~~booster~~***third*** vaccination. The selection of the preferred dose will be conditional on an acceptable safety profile for that dose.

Section 10.9 (Appendix 9: List of countries considered highly endemic for *Shigella*)

In this newly inserted Appendix, the following text has been added:

Africa

- ***Cameroon***
- ***Central African Republic***
- ***Chad***
- ***Democratic Republic of Congo***
- ***Eritrea***
- ***Ethiopia***
- ***Lesotho***
- ***Madagascar***
- ***Mali***
- ***Namibia***
- ***Niger***
- ***South Sudan***
- ***Togo***

Asia

- ***Bangladesh***
- ***India***
- ***Indonesia***

- ***Nepal***
- ***Pakistan***
- ***Tajikistan***

Oceania

- ***Papua New Guinea***

Section 11 (References)

The following references have been added:

Cohen D, Ashkenazi S, Green MS, et al. Double-blind vaccine-controlled randomised efficacy trial of an investigational Shigella sonnei conjugate vaccine in young adults. Lancet. 1997;175:1128-33.

Cohen D, Atsmon J, Artaud C, et al. Safety and immunogenicity of a synthetic carbohydrate conjugate vaccine against Shigella flexneri 2a in healthy adult volunteers: a phase 1, dose-escalating, single-blind, randomized, placebo-controlled study. Lancet Infect Dis. 2021;21:546-58.

Passwell JH, Ashkenazi S, Banet-Levi Y, et al. Age-related efficacy of Shigella O-specific polysaccharide conjugates in 1-4-year old Israeli children. Vaccine. 2010 28: 2231-35.

11. REFERENCES

BOOSTRIX (Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, adsorbed). Product information. February 2023.

Chung AW, Kumar MP, Arnold KB, et al. Dissecting polyclonal vaccine-induced humoral immunity against HIM using Systems Serology. Cell. 2015;163: 988-98.

Cohen D, Green MS, Block C, et al. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis. 1988;157:1068-71.

Cohen, D, Green M, Block C, et al. Reduction of transmission of shigellosis by control of houseflies (Musca domestica). Lancet. 1991;337:993-7.

Cohen D, Ashkenazi S, Green MS, et al. Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella* sonnei conjugate vaccine in young adults. Lancet. 1997;175:1128-33.

Cohen D, Atsmon J, Artaud C, et al. Safety and immunogenicity of a synthetic carbohydrate conjugate vaccine against *Shigella* flexneri 2a in healthy adult volunteers: a phase 1, dose-escalating, single-blind, randomized, placebo-controlled study. Lancet Infect Dis. 2021;21:546-58.

Cohen D, Ashkenazi S, Schneerson R, et al. Threshold protective levels of serum IgG to Shigella lipopolysaccharide: re-analysis of Shigella vaccine trials data. Clin Microbiol Infect. 2022;13:S1198-743X(22)00525-0.

Erlewyn-Lajeunesse M, Bonhoeffer J, Rüggeberg JU, Heath PT. Anaphylaxis as an adverse event following immunization. J Clin Pathol. 2007;60:737-9.

Ferreccio, C, Prado V, Ojeda A, et al., Epidemiologic patterns of acute diarrhea and endemic *Shigella* infections in children in a poor per urban setting in Santiago, Chile. Am J Epidemiol. 1991;134:614-27.

Global Burden of Disease (GBD) 2016 Diarrhoeal Disease Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018;18:1211-28.

INFANRIX HEXA (Diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis (inactivated), and Haemophilus influenzae type b vaccine). Product information. May 2023.

Launay O, Lewis DJM, Anemona A, et al. Safety profile and immunologic responses of a novel vaccine against *Shigella sonnei* administered intramuscularly, intradermally and intranasally: results from two parallel randomized Phase 1 clinical studies in healthy adult volunteers in Europe. EBioMedicine. 2017;22:164–72.

Launay O, Ndiaye AGW, Conti V, et al. Booster vaccination with GVGH *Shigella sonnei* 1790GAHB GMMA vaccine compared to single vaccination in unvaccinated healthy European adults: results from a Phase 1 clinical trial. Front Immunol. 2019;10:335.

Livio S, Strockbine NA, Panchalingam S, et al. *Shigella* isolates from the global enteric multicenter study inform vaccine development. Clin Infect Dis. 2014;59:933-41.

MENVEO (Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM197 Conjugate Vaccine). Product information. July 2023.

Micoli F, Rossi O, Conti V, et al. Antibodies elicited by the *shigella sonnei* GMMA vaccine in adults trigger complement-mediated serum bactericidal activity: results from a phase 1 dose escalation trial followed by a booster extension. Front Immunol. 2021;12:671325.

MR-VAC (Measles and rubella vaccine [live]). Product information. December 2021.

Necchi F, Saul A, Rondini S. Development of a high throughput method to evaluate serum bactericidal activity using bacterial ATP measurement as survival readout. PLoS One. 2017;12:e0172163.

Obiero CW, Ndiaye AGW, Sciré AS, et al. A Phase 2a randomized study to evaluate the safety and immunogenicity of the 1790GAHB generalized modules for membrane antigen vaccine against *Shigella sonnei* administered intramuscularly to adults from a shigellosis-endemic country. Front Immunol. 2017;8:1884.

Passwell JH, Ashkenazi S, Banet-Levi Y, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year old Israeli children. Vaccine. 2010 28:2231-35.

Riddle MS, Kaminsky RW, Di Paolo C, et al. Safety and immunogenicity of a candidate bioconjugate vaccine against *Shigella* flexneri 2a administered to healthy adults: a single-blind, randomized Phase I study. Clin Vaccine Immunol. 2016;23:908-917.

Rossi O, Molesti E, Saul A, Giannelli C, Micoli F, Necchi F. Intra-laboratory evaluation of luminescence based high-throughput serum bactericidal assay (L-SBA) to determine bactericidal activity of human sera against *Shigella*. High Throughput. 2020;9:14.

Rüggeberg JU, Gold MS, Bayas JM, et al. Brighton Collaboration Anaphylaxis Working Group. Anaphylaxis: case definition and guidelines for data collection, analysis, and presentation of immunization safety data. Vaccine. 2007;25:5675-84.

TYPHIM VI Salmonella typhi Vi Capsular Polysaccharide Vaccine. Product information. October 2021.

U.S. Department of Health and Human Services Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. Guidance for Industry- Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials; September 2007. (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical). *Accessed 15 December 2020*.

U.S. Department of Health and Human Services. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pe diatric Adverse Events. Corrected Version 2.1; July 2017. (https://rsc.niaid.nih.gov/clinical-research-sites/grading-severity-adult-pediatric-adverse-events-corrected-version-two-one). *Accessed 15 December 2020*.

World Health Organization. WHO COVID-19: Case Definitions; 16 December 2020. (https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.2). *Accessed 26 May 2021*.

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