A Multi-country, Multi-center, Open-labelled, Randomized, Controlled, Extended Phase III Clinical Trial to Evaluate the Immunogenicity and Tolerability of Sabin Strain Inactivated Poliovirus Vaccine Administered with or without Routine Infant Vaccines

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Statistical Analysis Plan

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Contents

1 List of Abbrevations

AE	Adverse Event
ANOVA	Anaysis of Variance
AR	Adverse Reaction
ASaT	All Subjects as Treated
ATC	Anatomic Therapeutic Chemical Classification
CI	Confidence Interval
CSR	Clinical Study Report
FAS	Full Analysis Set
FHA	Filamentous Hemagglutinin
GMC	Geometric Mean Concentration
GMFR	Geometric Mean Fold Rise
GMT	Geometirc Mean Titer
HepB	hepatitis B
IgG	Immunoglobulin G
ITT	Intent to Treat
LLoQ	Lower Limit of Qualification
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
PN	Preferred Name
PPS	Per Protocol Set
PRN	Pertactin
PT	Preferred Term
PT	Pertussis Toxin
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-COV-2	Severe Acute Respiratory Syndrome-Cornona Virus
SCR	Seroconversion Rate
SDTM	Study Data Tabulation Model
sIPV	Strain Inactivated Poliovirus Vaccine
SOC	System Organ Class
SPR	Sero-protection Rate
SS	Safety Set
TEAE	Treatment Emergent Adverse Event

ULoQ	Upper Limit of Qualification
WHODrug	World Health Organization Drug Dictionaries

2 Introduction

2.1 Preface

This document presents the Statistical Analysis Plan (SAP) for "A Multi-country, Multi-center, Open-labelled, Randomized, Controlled, Extended Phase III Clinical Trial to Evaluate the Immunogenicity and Tolerability of Sabin Strain Inactivated Poliovirus Vaccine Administered with or without Routine Infant Vaccines", which provides the details of methods to analyze baseline characteristics, immunogenicity and safety.

This SAP will be finalized and approved before the database is locked, and the programming of the corresponding statistical analysis will be completed until the database is locked.

Mockup Shell will be provided as an attachment of this SAP.

2.2 Purpose of Analysis

The purposes of the planned analysis described in this SAP are to evaluate the safety and immunogenicity of Sabin Strain Inactivated Poliovirus Vaccine(sIPV) administered with or without routine infant vaccines. The analysis results will be included in the Clinical Study Report (CSR), and may also be utilized for regulatory submissions, publications or others.

Additional analysis for other purposes, such as regulatory needs or sponsor requests, are not described in this SAP. If it occurs, the additional analyses are not included in the final CSR, but will be detailed in the document which presents the additional results.

2.3 Changes to Planned Analyses

The statistical analysis planned in this SAP is consistent with the ones in the protocol.

3 Objectives

3.1 Primary Objectives

(1) To evaluate the non-inferiority of immune response to polio vaccination, when administered concomitantly with routine vaccines.

(2) To evaluate the safety in terms of Adverse Reactions (ARs) (Vaccine-related Adverse Events (AEs)).

3.2 Secondary Objectives

(1) To evaluate non-inferiority of immune response to diphtheria and tetanus antigens, when routine vaccines are administered concomitantly with sIPV.

(2) To evaluate non-inferiority of immune response to acellular pertussis antigens, when routine vaccines are administered concomitantly with sIPV.

(3) To evaluate other immunogenicity against diphtheria, tetanus, acellular pertussis antigens.

(4) To evaluate other immunogenicity of sIPV, when administered concomitantly with routine vaccines.

(5) To evaluate the immunogenicity against hepatitis B and Hib, when routine vaccines are administered concomitantly with sIPV.

(6) To evaluate the immunogenicity against pneumococcal, when routine vaccines are administered concomitantly with sIPV.

(7) To evaluate other safety in terms of ARs (Vaccine-related AEs).

(8) To evaluate the safety in terms of Serious Adverse Events (SAEs).

4 Study Design

4.1 Overall Study Design

4.1.1 Overview of the Study

This is a multi-country, multi-center, open-labelled, randomized, controlled, extended phase Ⅲ clinical trial. Totally 1440 healthy infants of 6 weeks old (42-47 days) for Bangladesh and of 6-8 weeks old (42-56 days) for Pakistan (Bangladesh and Pakistan, each includes 720 participants) are planned to be enrolled, and then randomized in a 1:1:1:1 ratio into four groups, i.e., co-administration group 1 (group C1), co-administration group 2 (group C2), staggered administration group 1 (group S1) and staggered administration group 2 (group S2).

Participants in group C1 and C2 will receive sIPV at 6,10,14 weeks old (at 4-week intervals), administered concomitantly with routine infant vaccine (may include Adsorbed Diphtheria-Tetanus-whole cell Pertussis-Hepatitis B and Haemophilus influenza type b conjugate vaccine (DTP-HepB-Hib), PCV or rotavirus vaccine in accordance with the local routine vaccination schedule). Participants in group S1 will receive sIPV at 6,10,14 weeks old, and receive routine infant vaccines at 8,12,16 weeks old (at 4-week intervals, respectively). Participants in group S2 will receive routine infant vaccines at 6,10,14 weeks old, and receive sIPV at 8,12,16 weeks old (at 4-week intervals, respectively). The sample size allocations, vaccination schedules and blood collections for each group

in different countries are shown in the tables (table 1 to table 3) below.

Table 1 Study Design for Each Group

Group Code	Sample Size	Vaccination Schedule						Blood Collection Time	Antibody Tests Testing Item
		6 weeks	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks		
C1	360	sIPV+ Routine vaccines		sIPV+ Routine vaccines		sIPV+ Routine vaccines		Baseline 18 Weeks	Neutralizing antibody against polioviruses of type 1,2,3
C2	360	sIPV+ Routine vaccines		sIPV+ Routine vaccines		sIPV+ Routine vaccines		Baseline 18 Weeks	Antibodies against Diphtheria, Tetanus, Pertussis (FHA, Pertactin, Pertussis toxoid), Hepatitis B, Hib, Pneumococcal antibodies*
S1	360	sIPV	Routine vaccines	sIPV	Routine vaccines	sIPV	Routine vaccines	Baseline 18 Weeks	Neutralizing antibody against polioviruses of type 1,2,3
S2	360	Routine vaccines	sIPV	Routine vaccines	sIPV	Routine vaccines	sIPV	Baseline 18 Weeks	Antibodies against Diphtheria, Tetanus, Pertussis (FHA, Pertactin, Pertussis toxoid), Hepatitis B, Hib, Pneumococcal antibodies*
Total	1440								

*Only for serotype 1, 5, 6B, 14, 19F.

Table 2 Vaccination Schedule for Bangladesh

Groups	Vaccination Schedule					
	6 weeks	**8 weeks**	**10 weeks**	**12 weeks**	**14 weeks**	**16 weeks**
C1&C2	sIPV DTP-HepB-Hib PCV10		sIPV DTP-HepB-Hib PCV10		sIPV DTP-HepB-Hib PCV10	
S1	sIPV	DTP-HepB-Hib PCV10	sIPV	DTP-HepB-Hib PCV10	sIPV	DTP-HepB-Hib PCV10
S2	DTP-HepB-Hib PCV10	sIPV	DTP-HepB-Hib PCV10	sIPV	DTP-HepB-Hib PCV10	sIPV

Table 3 Vaccination Schedule for Pakistan

Groups	Vaccination Schedule					
	6 weeks	**8 weeks**	**10 weeks**	**12 weeks**	**14 weeks**	**16 weeks**
C1&C2	sIPV DTP-HepB-Hib PCV13 Rotavirus vaccine		sIPV DTP-HepB-Hib PCV13 Rotavirus vaccine		sIPV DTP-HepB-Hib PCV13	
S1	sIPV	DTP-HepB-Hib PCV13 Rotavirus vaccine	sIPV	DTP-HepB-Hib PCV13 Rotavirus vaccine	sIPV	DTP-HepB-Hib PCV13
S2	DTP-HepB-Hib PCV13 Rotavirus vaccine	sIPV	DTP-HepB-Hib PCV13 Rotavirus vaccine	sIPV	DTP-HepB-Hib PCV13	sIPV

4.1.2 Immunogencity Observation

About 3 ml venous blood will be collected before the first vaccination and 28 days (+7 days) after the last vaccination of sIPV or routine vaccines. Group C1 and S1 will be compared in terms of immunogenicity against polio, Group C2 and S2 will be compared in terms of immunogenicity against diphtheria, Tetanus, Pertussis, Hepatitis B, Hib and Pneumococcal.

4.1.3 Safety Observation

- For all participants, the immediate reactions within 30 minutes after each vaccination will be observed on site.
- Within 7 days after each vaccination, solicited selected AEs will be strictly recorded.
- Up to 28 days after the last dose vaccination, unsolicited AEs and any SAEs will be required to recorded.

4.2 Randomization

Stratified blocked randomization was carried out in this study, taking the study site as a stratification factor. The independent randomization statistician used SAS 9.4 to generate a randomization code list. In order to minimize the probability of relevant participants being informed of the group, the paper randomization card with numbers will be used. For each eligible participant, after enrollment, the grouping information will be revealed, including the name of the vaccine and the site of vaccination.

4.3 Sample Size Consideration

The sample size in this study is calculated based on the non-inferiority hypothesis of the immune response to sIPV, taking the combination vaccination group as the test group and staggered vaccination group as the control group. The sample size calculation parameters are as follows:

- The seroconversion rate of the control group is estimated as 90% referring to the previous clinical data of sIPV;
- The non-inferiority criterion is: the lower limit of 95% Confidence Interval (CI) of the difference between groups (test group-control group) >-10%;
- The allocation ratio between test group and control group for immunogenicity evaluation against either polio of three serotypes is 1:1;
- The one-sided significance level is 0.025;
- The overall statistical power (1-β) is 80%, with a corrected statistical power of 93.3% (1-20%/ 3) for statistical test of each serotype.

Considering the conditons above, the sample size calculated by using PASS 2022 (V22.0.2) for each group is 233. Considering a dropout rate of approximately 15% and a blood collection failure rate of approximately 10%, sample size of 360 is determined for each group.

5 Endpoints

5.1 Immunogenicity Endpoints

- **In the comparison between group C1 and group S1**, the immunogenicy endpoints for neutralizing antibody (Nab) against polioviruses of serotype I, II, III at 28 days after last vaccinaton of sIPV will be defined as follows, respectively:

(1) Seroconversion (4-fold increase) rate, which is defined as pre-vaccination Nab titer against polioviruses <1:8 and post-vaccination Nab titer against polioviruses ≥1:8, or 4-fold Nab titer against polioviruses increase in case that pre-vaccination Nab titer against polioviruses ≥1:8.

(2) Seroconversion Rate (SCR) in seronegative participants at pre-vaccination, which is defined as pre-vaccination Nab titer against polioviruses <1:8 and post-vaccination Nab titer against polioviruses≥1:8.

(3) 4-fold increase rate in seropositive participants at pre-vaccination, which is defined as 4-fold Nab titer against polioviruses increase in case that pre-vaccination Nab titer against polioviruses ≥1:8.

(4) Sero-Protection Rate (SPR), which is defined as Nab titer against polioviruses ≥ 1:8.

(5) Geometric Mean Titer (GMT) and Geometric Mean Fold Rise (GMFR), which is defined as the increase of Nab titers against polioviruses after vaccination compared to baseline.

(6) GMT and GMFR in seronegative participants at pre-vaccination

(7) GMT and GMFR in seropositive participants at pre-vaccination

- **In the comparison between group C2 and group S2**, the immunogenicy endpoints for Immunoglobulin G (IgG) antibody against Pertussis Toxin (PT), Filamentous Hemagglutinin (FHA) and Pertactin (PRN) at 28 days after last vaccinaton of DTP-HepB-Hib will be defined as follows, respectively:

(1) Seropositivity rate, which is defined as IgG antibody concentration after vaccination ≥20 EU/ml.

(2) SCR, which is defined as pre-vaccination IgG antibody concentration <5 EU/ml and post-vaccination concentration ≥20 EU/ml, or 5 EU/ml ≤ pre-vaccination IgG antibody concentration

<20 EU/ml and 4-fold concentration increase, or pre-vaccination IgG antibody concentration ≥20 EU/ml and 2-fold concentration increase.

(3) Geometric Mean Concentration (GMC) and GMFR, which is defined as the increase of IgG antibody concentrations after vaccination compare to baseline.

- **In the comparison between group C2 and group S2**, the immunogenicy endpoints for IgG antibody against diphtheria, tetanus, Hepatitis B (HepB), Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F) at 28 days after last vaccinaton of DTP-HepB-Hib will be defined as follows, respectively:

(1) SPR, which is defined as corresponding IgG antibody concentration after vaccination ≥cut off value.

(2) GMC and GMFR, which is defined as the increase of corresponding IgG antibody concentration after vaccination compare to baseline.

The cutoff values of above IgG antibodies are shown in Table 4.

Table 4 Cutoff Values of Different IgG Antibodies

Antibodies	Cutoff Values
Diphtheria	0.1 IU/ml
Tetanus	0.1 IU/ml
Hepatitis B	10 mIU/ml
Hib	0.15 μg/mL
Pneumococcal*	0.35 μg/mL

*The following serotypes will be detected in this study: 1, 5, 6B, 14, 19F

5.2 Safety Endpoints

(1) Adverse Events

- **Solicited Adverse Events:** including solicited local (vaccination site) and systemic AEs that occur during 7 days after each vaccination. The solicited local (vaccination site) AEs: including redness, swelling, rashes, induration and pruritus. The solicited systemic AEs: including fever, acute allergic reaction, diarrhea, decreased appetite, irritability, and decreased activity.
- **Unsolicited Adverse Events**: including all AEs except solicited AEs during the solicitation period, and all AEs during the non-solicitation period.

(2) Serious Adverse Events

6 Analysis Sets

(1) Full Analysis Set (FAS): Following the principle of Intention to Treat (ITT), it includes all participants who are randomized, complete at least one vaccination against the antigen to be evaluated, and have the valid immunogenicity results before the first vaccination. The participants who are vaccinated erroneously will be analyzed for the immunogenicity evaluation as randomized according to the ITT principle.

(2) Per Protocol Set (PPS): It is a subset of FAS which including all the participants who are eligible for this study according to the inclusion and exclusion criteria, and then randomized, complete full vaccination as protocol specified, and have valid immunogenicity results before and after the vaccination. Participants who meet the following conditions will be excluded from PPS:

- Those who has significant protocol deviations;
- Those who are vaccinated with the wrong vaccine;
- Use of protocol prohibited vaccines or drugs;
  - Other investigational or unlicensed products (drugs or vaccines);
  - Long-term use (lasting more than 14 days) of immunosuppressive or other immunomodulatory drugs (inhaled or topical steroids are allowed);
  - Immunoglobulin and/or blood preparations;
- Newly diagnosed autoimmune disease, including human immunodeficiency virus (HIV) infection;
- Other situations affecting the evaluation of vaccine immunogenicity.

For group C1 and S1, FAS and PPS will be defined for Nab against polioviruses of serotype I, II, III, respectively. For group C2 and S2, FAS and PPS will be defined for IgG antibodies of diphtheria, tetanus, pertussis (including PT, FHA and PRN), hepatitis B, Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F), respectively.

(3) Safety Set (SS): All randomized participants who completed at least one dose vaccination will be included in the safety set. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to All Subjects as Treated (ASaT) principle.

(4) Safety Set 1 for sIPV (SS1_sIPV) : All randomized participants who completed the 1st vaccination of sIPV will be included in SS1_sIPV. Participants who are vaccinated with the wrong

vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

(5) Safety Set 2 for sIPV (SS2_sIPV): All randomized participants who completed the 2nd vaccination of sIPV will be included in SS2_sIPV. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

(6) Safety Set 3 for sIPV (SS3_sIPV): All randomized participants who completed the 3rd vaccination of sIPV will be included in SS3_sIPV. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

(7) Safety Set 1 for sIPV and routine vaccines (SS1_sIPV&Routine): All randomized participants who completed 1st vaccination of sIPV and routine vaccines will be included in SS1_ sIPV&Routine. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

(8) Safety Set 2 for sIPV and routine vaccines (SS2_sIPV&Routine): All randomized participants who completed 2nd vaccination of sIPV and routine vaccines will be included in SS2_ sIPV&Routine. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

(9) Safety Set 3 for sIPV and routine vaccines (SS3_sIPV&Routine): All randomized participants who completed 3rd vaccination of sIPV and routine vaccines will be included in SS3_ sIPV&Routine. Participants who are vaccinated with the wrong vaccine will be analyzed for safety evaluation as treated according to ASaT principle.

7 Statistical Method

7.1 General Considerations

7.1.1 General Methods

(1) Descriptive Statistics

Unless otherwise specified, the following descriptive statistics are given according to the variable types:

- Continuous variables will be summarized by mean, standard deviation, median, minimum and maximum.
- Category or ordinal variables will be summarized by frequency counts and percentages, where the denominator will be the number of non-missing participants in the corresponding analysis set.

(2) Decimal Places

Unless otherwise specified, the number of decimal places in the TFLs will follow the belowing rules:

- The decimal place of minimum and maximum will be the same as the maximum one of raw data, and no more than 4.
- The decimal place of mean, median, standard deviation and 95% CI are one more than the maximum decimal place of raw data, and no more than 4.
- The decimal places of percentage and rate will be 2.
- If P value ≥0.0001, the decimal place will be 4, and if the P value <0.0001, it will be reported as "<0.0001".
- Statistics will be retained to 3 decimal places.
- The derived data will be retained to 2 decimal places.

7.1.2 Related Definitions and Derivation Rules

(1) Adverse Events Times

Occurrence time of AE (day) = Start date of AE – the corresponding vaccination date.

Duration of AE (day) = End date of AE – Start date of AE + 1.

(2) Treatment Emergent Adverse Event (TEAE)

The following rules will be used for TEAE.

- If the start date of AE is after (including) the date of the first vaccination, it will be defined as TEAE.
- If the start date of AE is before the date of first vaccination, it will be defined as non-TEAE.
- If either the start date of AE or the date of the first vaccination is missing such that it is not clear whether the AE occurred after the first vaccination, it will be defined as TEAE in all situations.

(3) Relationship of TEAE

- Related to the study vaccine means that the TEAE is "definitely related", "probably related" or "possibly related" to the study vaccine.
- Not related to the study vaccine means that the TEAE is “Not related” or “Unlikely related” to the study vaccine.

(4) Coding

Medical histories, AEs and SAEs will be coded by using the Medical Dictionary for Regulatory Activities (MedDRA) version 25.1 or later version. Prior and concomitant medications / vaccines will

be coded by using world health organization drug dictionaries 2022-Sep-1 (WHODrug_2022-Sep-1) or later version.

(5) Prior or Concomitant Medications

The following rules will be used to define prior and concomitant medications.

- If the end date of the medication is before (not including) the date of the first vaccination, it will be difined as a prior medication.
- If the start date of the medication is before (including) the date of the first vaccination and the end date of the medication is after (including) the date of the first vaccination, or the medication is still being used, or the start date of the medication is after (including) the date of the first vaccination, it will be defineded as a concomitant medication.
- If either the start date of the medication or the date of the first vaccination is missing such that it is not clear whether the medication occurred after the first vaccination, it will be defined as a concomitant medication in all situations.

(6) Prior or Concomitant Vaccines

The rules of prior and concomitant vaccines definition will be the same as the rules for prior or concomitant medication above.

(7) Immunogenicity Test Results

In the analysis of immunogenicity evaluation, "< LLoQ" will be treated as 1/2 of LLoQ (Lower Limit of Qualification), and "> ULoQ" will be treated as ULoQ (Upper Limit of Qualification).

7.1.3 The Window of Analysis

For visits after baseline, all analysis will be conducted based on the scheduled visits in the protocol. The unscheduled visits will not be included in the summary tables, but will be listed.

7.1.4 Analysis Software

All statistical analysis will be conducted by SAS 9.4 or later version.

7.1.5 Table and Listings

- **Tables**

Excluding safety evaluation, all data will be summarized by group (i.e., C1, C2, S1 and S2). In safety evaluation, the data will be summarized by combined group C (including C1 and C2) and combined group S (including S1 and S2).

- **Listings**

Unless otherwise specified, listings generally include participant ID and group. Study Data Tabulation Model (SDTM) data is preferred to display in the listing. They will be sorted by group, participant ID, visit or other relevant time (e.g., start date of AE).

7.2 Disposition of Participants

The number and percentage of participants in each category (screened, randomized, study complete and study discontinuation, the reasons of screened failure and discontiation, participants in each analysis set) will be summarized. Screened failure participants, discontinued participants and participants excluded from each analysis set will be listed, respectively.

7.3 Compliance

Descriptive statistics of whether completed each vaccination, whether completed the immunogenicity blood collection will be presented for each group.

Protocol deviations/violations by total and classification will be summarized by number of events, number of participants, and percentage of participants for each group. Fisher's exact test will be used to test the differences among groups. All protocol deviations/violations will be listed.

The above analysis will be analyzed based on all randomised participants.

7.4 Demographics and baseline Characteristics

Descriptive statistics will be used to summarize demographics and baseline variables as follows:

- **Demographics:** gender, race, ethnic and age.
- **Vital signs:** height, weight, axillary temperature, heart rate and breathing rate.
- **Clinical characteristics:** medical history, Severe Acute Respiratory Syndrome-Cornona Virus (SARS-CoV-2) rapid antigen test, prior medications/ vaccines.

Anaysis of Variance (ANOVA) will be used to compare continuous variables (including age, height, weight, axillary temperature, heart rate and breathing rate) among groups. Chi-square test/ fisher’s exact test will be used to compare categorical variables (incluing gender, race, ethinc, whether has

medical history and results of SARS-CoV-2 test) among groups.

Medical histories will be summarized by number of events, number of participants, and percentage of participants, and tabulated by System of Class (SOC) and Preferred Term (PT). Fisher's exact test will be used to test the differences among groups.

Demographics, vital signs, medical history and results of SARS-CoV-2 rapid antigen tests will be listed, respectively.

The above analysis will be analyzed based on FAS and PPS.

7.5 Prior and Concomitant Medications/ Vaccines

Prior and concomitant medications will be summarized by number of events, number of participants, and percentage of participants, and tabulated by Anatomic Therapeutic Chemical classification (ATC2) and Preferred Name (PN). Fisher's exact test will be used to test the differences among groups.

Prior and concomitant vaccines will be summarized by number of events, number of participants, and percentage of participants, and tabulated by ATC4 and PN. Fisher's exact test will be used to test the differences among groups.

The prior and concomitant medications/ vaccines will be listed.

The prior medications and vaccines will be analyzed based on FAS and PPS. The concomitant medications and vaccines will be analyzed based on SS.

7.6 Study Hypothesis

The following research hypothesis will be considered for the immunogenicity evaluation of **group C1 and group S1**:

- **Hypothesis test I**: At 28 days after full vaccination, group C1 will have a non-inferior seroconversion (4-fold increase) rate of Nab against polioviruses to group S1, ie:

$$\text{Null hypothesis } H_{01}: \pi_{C1j,d28}-\pi_{S1j,d28}\leq\Delta_1$$

$$\text{Alternative hypothesis } H_{11}: \pi_{C1j,d28}-\pi_{S1j,d28}>\Delta_1$$

Where j= 3 serotypes of Nab against polioviruses (including type 1, 2 and 3). $\pi_{C1j,d28}$, and $\pi_{S1j,d28}$ represent the seroconversion (4-fold increase) rate of type-*j* Nab against polioviruses at 28 days after full vaccination in group C1 and group S1, respectively, with the non-inferiority criterion Δ_1 is -10%, and a one-sided α is 0.025.

7.7 Immunogenicity Evaluation

- **Immunogenicity evaluation for Nab against polioviruses**

The following analysis will be performed for Nab against polioviruses of serotype I, II, III between **group C1 and group S1 at 28 days after last vaccination of sIPV**, respectively:

The seroconversion (4-fold increase) rate in all participants, SCR in seronegative participants at pre-vaccination, 4-fold increase rate in seropositive participants at pre-vaccination and SPR in all participants will be estimated in each group. Clopper Pearson method will be used to calculate 95% CIs and Cochran-Mantel-Haenszel-χ^2 (CMH-χ^2) test stratified by study site (including Bangladesh 01, Pakistan 01 and Pakistan 02) will be used to test the differences between the two groups. The CMH method will be used to calculate the rate difference between (group C1 - group S1) and 95% CIs. Non-inferiority will be concluded if the lower limit of the 95% CI of SCR difference between group C1 and Group S1 >-10%.

Analysis of covariance model will be modeled to analyze Nab level against polioviruses at 28 days after last vaccination of sIPV. In this model, the log-transformed Nab data against polioviruses at 28 days after last vaccination of sIPV will be included as dependent variable, the log-transformed Nab data before vaccination as a covariate, and treatment group (including C1 and S1) and study site (including Bangladesh 01, Pakistan 01 and Pakistan 02) are the fixed effect. From the model, the LS-adjusted log-transformed Nab level against polioviruses at 28 days after last vaccination of sIPV in each group and the difference between (group C1 - group S1) will be calculated. The adjusted GMT at 28 days after last vaccination of sIPV in each group, GMT ratio between (group C1/group S1), and its 95% CIs will be obtained after inverse log-transformation.

Descriptive statistics, including geometric mean and 95%CIs, for GMT and GMFR in all participants, seronegative participants at pre-vaccination and seropositive participants at pre-vaccination will be presented by groups. The group t-test after log-transformation will be used to compare the differences between groups. The ratio of GMT between groups and their 95% CIs will be estimated as well.

The forest plot of the post-vaccination seroconversion (4-fold increase) rate for all participants in comparison (group C1 and group S1) will be plotted.

The reverse cumulative distribution plot of antibody titers at pre- and post-vaccination time points will be plotted.

The above analysis will be based on the FAS and PPS.

- **Immunogenicity evaluation for IgG antibodies against pertussis**

The following analysis will be performed for IgG antibodies against PT, FHA and PRN of **group C2 and group S2 at 28 days after last vaccinaton of DTP-HepB-Hib**, respectively:

The seropositivity rate and SCR for all participants will be estimated by groups. The Clopper Pearson method will be used to calculate 95% CIs and the CMH-χ^2 test considering the study site (including Bangladesh 01, Pakistan 01 and Pakistan 02) as a stratification factor will be used to test the differences between groups. The CMH-χ^2 method will be used to calculate the rate difference between (group C2 - group S2) and 95% CIs.

Analysis of covariance model will be modeled to analyze IgG antibody level against PT, FHA and PRN at 28 days after last vaccination of DTP-HepB-Hib. In this model, the log-transformed IgG antibody data against PT, FHA and PRN at 28 days after last vaccination of DTP-HepB-Hib will be included as dependent variable, the log-transformed IgG antibody data before vaccination as a covariate, and treatment group (including C2 and S2) and study site (including Bangladesh and Pakistan) are the fixed effect. From the model, the LS-adjusted log-transformed IgG antibody level against PT, FHA and PRN at 28 days after last vaccination of DTP-HepB-Hib in each group and the difference between (group C2 - group S2) will be calculated. The adjusted GMC at 28 days after last vaccination of DTP-HepB-Hib in each group, GMC ratio between (group C2/group S2), and its 95% CIs will be obtained after inverse log-transformation.

Descriptive statistics, including geometric mean and 95% CIs, for GMC and GMFR of all participants will be presented by groups. The grouped t-test after log-transformation will be used to compare the differences between groups. The ratio of GMC between groups and their 95% CIs will be estimated as well.

The reverse cumulative distribution plot of antibody concentrations at pre- and post-vaccination time points will be plotted.

The above analysis will be based on the FAS and PPS.

- **Immunogenicity evaluation for IgG antibodies against diphtheria, tetanus, HepB, Hib and pneumococcal**

The following analysis will be performed for IgG antibodies against diphtheria, tetanus, HepB, Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F) of **group C2 and group S2 at 28 days after last vaccination of DTP-HepB-Hib and PCV**, respectively:

The SPR for all participants will be estimated by groups. The Clopper Pearson method will be used to calculate 95% CIs and the CMH-χ^2 test considering the study site (including Bangladesh 01, Pakistan 01 and Pakistan 02) as a stratification factor will be used to test the differences between groups.

Analysis of covariance model will be modeled to analyze IgG antibodies against diphtheria, tetanus, HepB, Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F) at 28 days after last vaccination of DTP-HepB-Hib. In this model, the log-transformed IgG antibodies against diphtheria, tetanus, HepB, Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F) at 28 days after last vaccination of DTP-HepB-Hib will be included as dependent variable, the log-transformed IgG antibody data before vaccination as a covariate, and treatment group (including C2 and S2) and study site (including Bangladesh and Pakistan) are the fixed effect. From the model, the LS-adjusted log-transformed IgG antibodies against diphtheria, tetanus, HepB, Hib and pneumococcal (including serotypes of 1, 5, 6B, 14 and 19F) at 28 days after last vaccination of DTP-HepB-Hib in each group and the difference between (group C2 - group S2) will be calculated. The adjusted GMC at 28 days after last vaccination of DTP-HepB-Hib in each group, GMC ratio between (group C2/group S2), and its 95% CIs will be obtained after inverse log-transformation.

Descriptive statistics, including geometric mean and 95% CIs, for GMC and GMFR of all participants will be presented by groups. The grouped t-test after log-transformation will be used to compare the differences between groups. The ratio of GMC between groups and their 95% CIs will be estimated as well.

The reverse cumulative distribution plot of antibody concentrations at pre- and post-vaccination time points will be plotted.

The above analysis will be based on the FAS and PPS.

7.8 Safety Evaluation

7.8.1 Adverse Events

The following analysis will be based on SS. The analysis of AEs for each vaccination of sIPV will be based on SS1_sIPV, SS2_sIPV, SS3_sIPV and the analysis of AEs for each vaccination of sIPV and

routine vaccines will be based on SS1_sIPV&Routine, SS2_sIPV&Routine, SS3_sIPV&Routine, respectively.

All AEs will be coded by using MedDRA 25.1 or later version, and be summarized by SOC and PT.

This study focuses on the analysis of AEs occurring during the vaccination, which includes TEAEs occurring after (including) the first vaccination and within 28 days after the full vaccination. AEs which occurred before the first vaccination and 28 days after the full vaccination will be listed. AEs below are those that occurred during the vaccination if not otherwise specified.

The number of events, number of participants and percentage of participants reported the AEs will be calculated by groups (combined group C and combined group S), fisher's exact test will be used to test the differences between groups. The AEs will be summarized as following:

- ✓ All AEs;
- ✓ Vaccine-related AEs;
- ✓ Vaccine-unrelated AEs;
- ✓ AEs by severity (grade 1, grade 2, grade 3, grade 4, grade 5, ≥grade 2 and ≥grade 3);
- ✓ AEs by severity, related to vaccine;
- ✓ AEs by severity, unrelated to vaccine;
- ✓ AEs by onset time (30 minutes, 0-7 days, 8-28 days, 0-28 days, after 28 days);
- ✓ AEs by onset time, related to vaccine;
- ✓ AEs by onset time, unrelated to vaccine;
- ✓ AEs by doses；
- ✓ AEs by doses, related to vaccine；
- ✓ AEs by doses, unrelated to vaccine；
- ✓ AEs leading to early termination；
- ✓ AEs leading to early termination, related to vaccine.

Fisher's exact test will be used to compare the difference among groups.

When one participant experiences more than one AEs, he/she will be counted once to calculate the incidence, and the severest and most related events will be included when analyze the severity and relationship to the vaccine of AEs.

All AEs and AEs leading to early termination will be listed.

7.8.2 Serious Adverse Events

The number of events, number of participants and percentage of participants reported the SAEs will be calculated by groups. Fisher's exact test will be used to compare the difference among groups. The SAEs will be summarized as following:

- ✓ All SAEs;
- ✓ Vaccination-related SAEs;
- ✓ Vaccination-unrelated SAEs.

All SAEs will be listed.

7.9 Handling of Missing Data

- The missing values of immunogenicity analysis in FAS will be imputed by using Last Observation Carried Forward (LOCF) method.
- The missing values of safety evaluation will not be imputed in this study.

7.10 Subgroup Analysis

Subgroup analysis is planned for both immunogenicity evaluation and safety evaluation in participants of different study country (including Bangladesh and Pakistan).

7.11 Multiplicity

The study hypothesis in chapter 7.6 will be achieved only when the non-inferiority of seroconversion (4-fold increase) rates against all poliovirus type I, II, III are all concluded. Therefore, there is no need to correct for type I error. But there is a need to correct for type II error, in order to ensure the overall statistical power (1-β) is 80%, a corrected statistical power of 93.3% (1-20%/ 3) for hypothesis test of each serotype is conducted.

7.12 Interim Analysis

No interim analysis is planned.

Version History

Version	Date	Author	Changes &Rationale
V1.0	2024-10-28	Yudou Huang	Initial Release