An Open-label Observation of the Bivalent Enterovirus Vaccine (Vero Cell), Inactivated in Healthy Adults and Children Aged 6-12 Years, and a Randomized, Double-Blind, Controlled Phase I/II Clinical Trial Evaluating Tolerability, Safety and Immunogenicity in Children Aged 6-71 Months

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1 Abbreviations and Definitions

AE	Adverse Event
ASaT	All Subjects as Treated
CI	Confidence Interval
FAS	Full Analysis Set
GMT	Geometric Mean Titer
ITT	Intention to Treat
LOCF	Last Observation Carried Forward
Max	Maximum
Mean	Mean
MedDRA	Medical Dictionary for Regulatory Activities
Median	Median
Min	Minimum
PPS	Per Protocol Set
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SS	Safety Set
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
WHODD	World Health Organization Drug Dictionary

2 Study Overview

2.1 Introduction

This document is the statistical analysis plan (SAP) for the following study: "**An Open-label Observation of the Bivalent Enterovirus Vaccine (Vero Cell), Inactivated in Healthy Adults and Children Aged 6-12 Years, and a Randomized, Double-Blind, Controlled Phase I/II Clinical Trial Evaluating Tolerability, Safety and Immunogenicity in Children Aged 6-71 Months**", which primarily describes the specific statistical analysis methods for participant baseline characteristics, immunogenicity and safety evaluation. The corresponding statistical analysis results of this study will be used for the registration application of this product.

This SAP will be finalized and approved before database lock, and the corresponding statistical analysis programming will be progressively refined as study data accumulates until database lock. The mockup shell for this SAP will be provided separately as an attachment.

2.2 Analysis Objective

The purpose of this SAP is to evaluate the safety of the Bivalent Enterovirus Vaccine (Vero Cell), Inactivated in healthy adults and children aged 6-12 years, as well as to evaluate tolerability, safety and immunogenicity in children aged 6-71 months. The corresponding statistical analysis results will be presented in the clinical summary report and will also be used for article publication and other clinical needs.

Post-hoc analysis may further explore the study data, but as they are unforeseeable, they are not described in this SAP. Should any such analyses be conducted subsequently, the corresponding statistical methods will be detailed in the final statistical analysis report or clinical study report.

Additional analyses for other purposes, such as publication, regulatory authority or sponsor requirements, are also unforeseeable and thus not described in this SAP. Should they occur subsequently, the corresponding statistical analysis methods for these additional analyses may not be detailed in the final clinical study report but will be elaborated in the document presenting the additional results.

2.3 Changes Compared to the Protocol

The statistical analyses stated in this SAP are consistent with those required by the protocol.

3 Study Objectives

3.1 Phase I

To evaluate the tolerability and safety of the investigational vaccine in participants of different age groups.

3.2 Phase II

To evaluate the immunogenicity, immune persistence and safety of the investigational vaccine in participants of different age groups.

4 Trial Design

4.1 Overall Design

The Phase I clinical trial is conducted in two parts: Part 1 follows an open-label design and Part 2 follows a randomized, double-blind, active-controlled design. The Phase II clinical trial follows a

randomized, double-blind, active-controlled design.

4.1.1 Phase I

Phase I participants are stratified into four age strata: 18-59 years, 6-12 years, 24-71 months and 6-23 months.

- **Part 1**

Part 1 plans to enroll 36 healthy adults aged 18-59 years and 36 children aged 6-12 years. Two doses of the investigational vaccine will be administered sequentially according to a 0, 1-month schedule, ascending from low dose [redacted], to medium dose [redacted] to high dose [redacted] Adults will be enrolled before children aged 6-12 years (sequential enrollment). In both adults and children aged 6–12 years, 12 participants will be assigned to each of the different dose groups.

- **Part 2**

Part 2 plans to enroll 72 healthy children aged 6-71 months, who will be vaccinated according to the 0, 1-month schedule and dosed sequentially from low, to medium, to high dose. Each dose stage adopts a randomized, double-blind, active-controlled design. At each dose stage, 12 participants aged 6-23 months and 12 participants aged 24-71 months will be enrolled and assigned in a 3:1 ratio to receive either the test vaccine or the comparator (EV71 vaccine).

The dose-escalation principle within each age group is as follows: dosing proceeds from low, to medium, to high dose. Escalation to a higher dose stage may proceed only after the 0-7 day safety observation following the first dose at the lower-dose stage has been completed and confirmed to be safe, as assessed by adverse events and the results of routine blood, blood biochemistry and routine urine testing (where applicable). The sequential enrollment principle across different age groups is as follows: from adults to children aged 6–12 years, and then to children aged 6–71 months. Enrollment of participants in the 6–12 years age group for a given dose level may only occur after the 0–7 day safety observation following the first dose in adults at the same dose level has been completed and safety has been confirmed. Furthermore, vaccination of the 6–71 months age group may only begin after the 7-day safety observation following the second dose in the adult medium-dose group has been completed.

The overall design for Phase I trial is shown in Table 1:

Table 1 Phase I Trial Design for Bivalent Enterovirus Vaccine (Vero Cell), Inactivated

Design	Age Group	Total	Comparator Vaccine	Test Vaccine (per dose level)			Vaccination schedule (months)	Urinalysis / complete blood count / blood biochemistry test timepoint*
				Low	Medium	High		
Part 1 (open-label)	18-59 years	36	-	12	12	12	0,1	D0,3,30,33
	6-12 years	36	-	12	12	12		
Part 2 (randomized, double-blind, controlled)	6-23 months	36	9	9	9	9		-
	24-71 months	36	9	9	9	9		D0,3,30,33
Total		144	18	42	42	42	-	-

** In the 6-71 months group, only participants aged 24-71 months will be tested prior to the first dose. Whether tests will be performed on D3 (V2), D30 (V5) and D33 (V6) will depend on the corresponding test results in the 6-12 years group. If, after the first dose at any dose stage in the 6-12 years group, 10% of participants show vaccine-related abnormal and clinically significant test results, then blood and urine samples will be collected on D3 (V2), D30 (V5) and D33 (V6) for testing in the corresponding dose stage of the 24-71 months group.*

4.1.1.1 Safety Observation

- **Adverse Events (AE) and Serious Adverse Events (SAE)**

All AEs occurring within 30 minutes after each dose, solicited AEs on Days 0-7, unsolicited AEs on Days 0-30, all SAEs from the first dose through 6 months after the full vaccination schedule, and all pregnancy events from the first dose through 3 months after the full vaccination schedule will be collected for all participants.

- **Laboratory Examinations**

Approximately 5.5 ml of blood (about 2.0 ml anticoagulated and 3.5 ml non-anticoagulated) and approximately 2-10 ml of urine will be collected from adults and 6-12 year-old children before each dose and on Day 3 after each dose, for complete blood count, blood biochemistry and urinalysis testing to evaluate vaccine safety. In the 6–71 months age group, the above tests are performed only in participants aged 24–71 months prior to the first dose.

- **Urine Pregnancy Test**

A urine pregnancy test will be performed in female participants of childbearing potential (post-menarche, pre-menopause, or in accordance with local determination criteria) prior to each dose.

4.1.2 Phase II

Phase II plans to enroll 600 children aged 6-71 months (360 aged 6-23 months and 240 aged 24-71 months), who will be randomized in a 1:1:1:1 ratio to a low-dose [redacted], medium-dose [redacted] or high-dose [redacted] test group or to the control group. Two doses of the test vaccine or the comparator will be administered according to the 0, 1-month schedule.

The overall design for Phase II trial is shown in Table 2:

Table 2 Phase II Trial Design for Bivalent Enterovirus Vaccine (Vero Cell), Inactivated

Age group (months)	Total	Comparator vaccine	Test vaccine (per dose level)			Vaccination schedule (months)	Blood-sampling timing (days)
			Low	Medium	High		
6~23	360	90	90	90	90	0,1	D0,60,210
24~71	240	60	60	60	60		
Total	600	150	150	150	150	-	-

4.1.2.1 Safety Observation

All AEs occurring within 30 minutes after each dose, solicited AEs on Days 0-7, unsolicited AEs on Days 0-30, all SAEs from the first dose through 6 months after the full vaccination schedule will be collected for all participants.

4.1.2.2 Immunogenicity Blood Sampling

Approximately 3.0 ml of venous blood will be collected from each participant on Days 0, 60 and 210. The serum obtained will be used to test EV71 and CA16 neutralizing antibodies in order to evaluate vaccine immunogenicity and immune persistence.

4.2 Randomization

Part 1 of the Phase I trial (adults and 6-12 year-old children groups) follows an open-label design, with 12 doses of test vaccine prepared at each dose level.

Part 2 of the Phase I trial (the 6-71 month-old children group) and the entire Phase II trial follow a randomized, double-blind design. An independent third-party randomization statistician will use SAS software (version 9.4) and apply a stratified block randomization method, with age group (6-23 months, 24-71 months) as the stratification factor, to assign participants to test and control groups in accordance with the protocol, and to generate a participant randomization list. Vaccines are numbered according to the participant randomization list (each vaccine has a unique identifier).

4.3 Sample Size

In accordance with the requirements of the "Drug Registration Management Measures", "Good Clinical Practice for Drugs", " Vaccine Clinical Trial Technical Guiding Principles", "Guiding Principles for Quality Management of Vaccine Clinical Trials (Trial)", etc., and taking into consideration the pharmaceutical-process characteristics and pre-clinical evaluation results of the vaccine, the sample sizes for Phase I and Phase II are allocated as follows:

Phase I clinical trial is a small-scale study (20-30 participants), focusing on evaluating the safety of the vaccine. The sample size for this trial is not calculated based on any statistical hypothesis but is determined according to the general requirements for Phase I clinical trials. A total of 144 participants will be enrolled, with 36 participants each allocated to the following age groups: adults, children aged 6–12 years, children aged 24–71 months, and children aged 6–23 months. For children aged 6-71 months, 24 participants will be assigned to test group and 18 to the control group at each dose level.

Phase II Clinical Trial is intended to evaluate the immunogenicity and safety of the vaccine in the target population, with a minimum sample size of 300 participants. The sample size for this trial is 600 participants, consisting of 450 in the three test dose groups in total, and 150 in the control group.

5 Evaluation Endpoints

5.1 Immunogenicity Endpoints

5.1.1 Primary Immunization Period

The following immunogenicity endpoints are defined for the neutralizing antibodies against EV71 and CA16 in Phase II participants at 30 days after the full vaccination separately:

(1) Post-vaccination seroconversion rate: either a change from seronegative to seropositive for neutralizing antibodies after vaccination in individuals who are seronegative at baseline; or a ≥4-fold

rise in neutralizing antibody titer after vaccination in individuals who are seropositive at baseline.

(2) Post-vaccination seroconversion rate in baseline seronegative population: Baseline seronegative population is defined as those with negative neutralizing antibody results prior to vaccination; Seroconversion in the baseline seronegative population is defined as those with pre-vaccination neutralizing antibodies negative and post-vaccination neutralizing antibodies positive.

(3) Post-vaccination seroconversion rate (≥4-fold rise rate) in baseline seronegative population: Baseline seropositive population is defined as those with positive neutralizing antibodies results prior to vaccination; Seroconversion in the baseline seropositive population is defined as those with pre-vaccination neutralizing antibodies positive, whose post-vaccination neutralizing antibodies titer achieves a 4-fold or greater increase compared to pre-vaccination.

(4) Post-vaccination Seropositive rate.

(5) Post-vaccination Geometric Mean Titer (GMT) and Geometric Mean Increase (GMI).

(6) Post-vaccination GMTand GMI in baseline seronegative participants.

(7) Post-vaccination GMT and GMI in baseline seropositive participants.

5.1.2 Immune Persistence Follow-up

The immune persistence endpoint for neutralizing antibodies against EV71 and CA16 at 6 months after full vaccination for Phase II participants are defined separately as follows:

(1) Post-vaccination Seropositive rate.

(2) Post-vaccination GMT .

(3) Post-vaccination GMT in baseline seronegative participants.

(4) Post-vaccination GMT in baseline seropositive participants.

5.2 Safety Endpoints

<u>The following safety endpoints are defined for Phase I and Phase II participants, respectively:</u>

(1) Adverse Events

(1.1) Solicited Adverse Events: Refers to injection site (local) and non-injection site (systemic) adverse events occurring during the solicitation period (0-7 days after each vaccination).

- **Participants aged 6-23 months**

✧ Local (Injection Site) Adverse Events: Injection Site Tenderness, Injection Site Induration,

Injection Site Swelling, Injection Site Erythema, Injection Site Rash, Injection Site Pruritus.

✧ Systemic (Non-Injection Site) Adverse Events: Acute allergic reaction, Diarrhoea, Anorexia, Vomiting, Irritability, Decreased activity, Fever (axillary temperature).

➢ **Participants aged 24-71 months**

✧ Local (Injection Site) Adverse Events: Injection Site Pain, Injection Site Induration, Injection Site Swelling, Injection Site Erythema, Injection Site Rash, Injection Site Pruritus.

✧ Systemic (Non-Injection Site) Adverse Events: Acute allergic reaction, Diarrhoea, Anorexia, Vomiting, Nausea, Fatigue, Fever (axillary temperature).

➢ **Children aged 6-12 years and adults aged 18-59 years**

✧ Local (Injection Site) Adverse Events: Injection Site Pain, Injection Site Induration, Injection Site Swelling, Injection Site Erythema, Injection Site Rash, Injection Site Pruritus.

✧ Systemic (Non-Injection Site) Adverse Events: Acute allergic reaction, Diarrhoea, Anorexia, Vomiting, Nausea, Fatigue, Muscle pain (non-injection site), Headache, Cough, Fever (axillary temperature).

(1.2) Unsolicited Adverse Event: include unsolicited symptoms occurring during the solicitation period and all symptoms occurring outside the solicitation period.

(2) Serious adverse events.

(3) Laboratory examinations (applicable only to adults and 6-12 years children in the Phase I trial), including:

➢ Routine blood: white blood cell count, hemoglobin, platelet count.

➢ Blood biochemistry: alanine aminotransferase, aspartate aminotransferase, total bilirubin, creatinine, fasting blood glucose.

➢ Routine urine: urinary protein.

6 Analysis Sets

The following immunogenicity analysis sets are defined for Phase II participants:

(1) Full Analysis Set (FAS): Following the Intention-to-Treat Analysis (ITT) principle, all participants who have been randomized, received at least 1 dose of the trial vaccination, completed at least one blood collection (either pre or 30 days post full-immunization), and have valid antibody titer values

will be included. Participants with vaccination errors will be evaluated for immunogenicity according to their original randomization group based on the ITT principle.

(2) Per Protocol Set (PPS): A subset of the FAS, including all participants who meet the inclusion criteria, do not meet the exclusion criteria, randomized, completed vaccination and blood sample collection before vaccination and 30 days after full vaccination within the protocol-specified time windows and have valid antibodies titer values. Participants with protocol deviations that could significantly impact the immunogenicity analysis should be excluded.

(3) Immune Persistence Set (IPS): Including all participants who completed two doses of vaccination, completed blood collection 6 months after the full immunization within the specified time window, and have valid antibody titer values.

FAS and PPS are primarily used for the 30-day post-full-vaccination immunogenicity evaluation and baseline-characteristic evaluation; IPS is primarily used for immunogenicity evaluation at 6 months post full immunization. In this trial, FAS, PPS, and IPS are defined for EV71 and CA16 neutralizing antibodies, respectively.

The Safety Analysis Set is defined separately for Phase I and Phase II participants as follows:

(4) Safety Set (SS): Including all participants who have been (randomly) enrolled and received at least one dose of the trial vaccination. Among them, according to the ASaT (All Subjects as Treated) principle, participants with vaccination errors will be analyzed based on the actual vaccine group administered.

The safety set is divided into the overall safety analysis set, the first-dose safety analysis set, and the second-dose safety analysis set. The safety analysis for each dose is performed based on the actual number of participants vaccinated for that dose. The first-dose safety set includes all participants who completed the first dose of vaccination, denoted as SS1; the second-dose safety analysis set includes all participants who completed the second dose of vaccination, denoted as SS2.

The safety set is used for the safety evaluation of the study, and the safety set for each dose is used for the safety evaluation of each dose.

The above analysis set will be determined through joint discussion by the principal investigator, sponsor, statistician, and data management team during the data blind review meeting prior to database lock.

7 Statistical Analysis Methods

7.1 General Consideration

7.1.1 General Analytical Methods

- **Descriptive Statistics**

Unless otherwise specified, the following descriptive statistics will be provided based on the variable type:

- ✓ Continuous variables will be summarized using Mean, Standard Deviation (SD), Median, Maximum (Max), and Minimum (Min). For immunogenicity evaluations and laboratory test indicators, Q1 and Q3 may also be used.
- ✓ Categorical or ordinal variables will be summarized using frequency and frequency (percentage), where the denominator for percentage calculation is the number of non-missing participants in the corresponding analysis set
- ✓ Titers and their fold increases will also be analyzed using the following descriptive statistics: geometric mean and 95% confidence interval. Geometric mean titers are obtained by performing an inverse logarithmic transformation on the mean of the logarithmized original titers. The 95% confidence interval is derived as follows: the 95% confidence interval for the mean of the logarithmized original titers is constructed using the T-distribution, after which the upper and lower bounds of this interval are separately subjected to inverse logarithmic transformation to obtain the 95% confidence interval for titers on the original scale. The analytical method for the fold increase in titers is similar.

- **Decimal Places**

Unless otherwise specified, the number of decimal places in the analysis report shall follow the rules below:

- ✓ Report minimum, maximum with the same decimal places as the original data (not exceeding 4 digits).
- ✓ Mean, geometric mean, median, standard deviation, and 95% confidence intervals should be reported with one more decimal place than the maximum number of decimal places of the original data, but not exceeding four decimal places.
- ✓ Percentages, rates, and rate differences are retained to two decimal places;

- ✓ If the P value is ≥0.0001, retain it to four decimal places; if the P value is <0.0001, report it as "<0.0001";
- ✓ All statistical test statistics are retained to three decimal places;
- ✓ Derived data are retained to two decimal places.

7.1.2 Relative Definitions and Derivative Rules

- ➢ **Baseline**

Unless otherwise specified, the Baseline is defined as the last non-missing measurement value prior to the first dose vaccination.

- ➢ **Adverse Events**

Time to Adverse Event Onset (days) = Adverse event start date - Vaccination date of the corresponding dose associated with the adverse event.

Duration (days) = Adverse event end date - Adverse event start date + 1.

- ➢ **Treatment Emergent Adverse Event (TEAE)**

Treatment Emergent Adverse Event (TEAE) is defined as adverse event that occurs after/on the first vaccination. The programming follows the rules below:

- ✓ If the onset of the adverse event occurs on or after the date of the first vaccination, it is counted as a TEAE;
- ✓ If the onset of the adverse event occurs before the date of the first vaccination, it is classified as a non-TEAE
- ✓ If the onset date of the adverse event or the date of the first vaccination is missing, such that it cannot be definitively determined whether the event occurred after the first vaccination, the adverse event is classified as a TEAE.

- ➢ **Relationship to the Study Vaccine**

"Related to the investigational vaccine" refers to adverse events for which the association with the investigational vaccine is assessed as "certainly related," "probably related," or "possibly related."

"Not related to the investigational vaccine" refers to adverse events for which the association with the investigational vaccine is assessed as " unlikely related" or " not related."

- ➢ **Prior/Concomitant Medication**

The determination of Prior/Concomitant medications follows the rules below:

- ✓ Medications whose use ended before (not including) the day of the first vaccination are recorded as prior medication.
- ✓ Medications that started or ended on or after the day of the first vaccination, medications used continuously, or medications with unclear timing are recorded as concomitant medication.

➢ **Prior/Concomitant Vaccine**

The judgment rules are the same as those for prior/concomitant medications.

➢ **Coding**

Prior/current medical history, non-drug therapy, adverse events and serious adverse events will be coded according to the MedDRA version 26.1 or higher, and classified and summarized by the System Organ Class (SOC) and Preferred Term (PT) levels. Prior/concomitant medications and prior/concomitant vaccines will be coded according to the World Health Organization Drug Dictionary - 2023 Q3 or higher version. Prior/Concomitant Medications will be classified and summarized according to the Anatomical Therapeutic Chemical Classification 2 (ATC2) and the standard drug name.

➢ **Immunogenicity test results**

In the immunogenicity analysis, for EV71 and CA16 neutralizing antibodies, Results reported as "negative" will be assigned with a value of 1 and then included in the analysis.

7.1.3 Analysis Window

For visits after baseline, when analyzed by visit, analysis will be performed according to the protocol-scheduled visit time points; time points outside the protocol plan will not be considered. Examination results outside the protocol schedule will be shown in listings.

7.1.4 Analysis Software

All statistical analyses will be performed using the statistical software SAS version 9.4 or higher.

7.1.5 Tables and Listings

➢ **Tables**

Data are generally summarized by group (Phase I part 1: low-dose test group, medium-dose test group, and high-dose test group; Phase I part 2 and Phase II: low-dose test group, medium-dose test group,

high-dose test group, and control group). The groups are generally presented in columns.

- **Listings**

Unless otherwise specified, all listings will include screening/study number, group and will prioritize the display of original data from the Study Data Tabulation Model (SDTM). Listings are generally sorted sequentially by group, screening/study number, visit date, or other related date (such as AE onset date).

7.2 Participant Disposition

The numbers of participants screened, (randomly) enrolled, completed the study, as well as the number of participants entering each analysis set will be summarized for Phase I and Phase II separately. The reasons for screening failure and reasons for early trial withdrawal will be analyzed.

List the screening failure participants, withdrawal participants, and participants not included in each analysis set, respectively.

7.3 Compliance

Descriptive statistics will be performed on the compliance of participants in each group for Phase I and Phase II (including completion of 1st dose of vaccination, completion of 2nd dose of vaccination, completion of full course of vaccination, completion of blood collection at 30 days after full immunization (Phase II only), and completion of blood collection at 6 months after full immunization (Phase II only)). Chi-square test/Fisher's exact test will be used for statistical testing among groups.

The number of events, the number of affected participants, and the corresponding incidence of protocol deviations will be calculated for each age group in Phase I and II separately. Use the Fisher's exact test to perform statistical testing for differences. Provide a list of protocol deviation.

The above analysis will be based on all (randomly) enrolled participants.

7.4 Demographic and Baseline Characteristics

Except for vital signs (analyzed only for Phase I participants aged 18–59 years), conduct the following statistical descriptions for the demographic and baseline characteristics of participants in each age group for Phase I and Phase II respectively:

- Demographic data: including age (years/months), Sex, Ethnicity;
- Physical examination: including height, weight, skin examination, body temperature, pharyngeal

examination.

- Vital Signs: including diastolic blood pressure, systolic blood pressure, heart rate, respiratory rate);
- Screening period clinical characteristics (including presence or absence of prior/current medical history)

Based on the distribution characteristics of the variables, Analysis of Variance (ANOVA) will be used to statistically compare the group differences for age (years/months), height, weight, systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate. Chi-square Test/Fisher's exact test will be used to statistically compare the group differences for sex, ethnicity, skin examination, pharyngeal examination, prior/current medical history, as well as urine pregnancy test results.

Prior/current medical history will be coded using MedDRA 26.1 or higher version, classified and counted according to SOC and PT levels. The number of occurrences, number of participants, and incidence of prior/current medical history for participants in each group will be calculated for total and each category. Fisher's exact probability test will be performed for statistical comparisons.

Prior medications will be coded using the WHODD 2023 Q3 or higher version. Prior medications will be classified and summarized according to ATC2 and standard drug names. The number of usage, number of participants, and usage rates for prior medications will be calculated for the total and each category. Statistical comparisons between groups will be conducted using Fisher's exact test.

Provide listing of demographic data, physical examination, vital signs, urine pregnancy examination, prior/current medical history, prior medication and prior vaccine, respectively.

The above analysis for Phase I will be based on SS while based on SS, FAS, and PPS for Phase II.

7.5 Concomitant Medication, Concomitant Vaccine and Non-drug Therapy

Concomitant medications will be coded using the WHOD 2023 Q3 or higher version. Concomitant medications will be classified and summarized by ATC Level 2 and standard drug name. The number of usage, number of participants, and usage rate for concomitant medications will be calculated overall and by category. Group comparisons will be performed using Fisher's exact test.

Concomitant vaccines will be classified according to the categories specified in the protocol. The number of usage, number of participants, and usage rate of concomitant vaccines in each group will be calculated both as total and by category. Fisher's exact test will be used to statistically compare differences between groups.

Non-drug therapy will be coded using MedDRA 26.1 or higher version, classified and counted according to SOC and PT levels. The number of usage, number of participants, and usage rate of non-drug therapy in each group will be calculated for total and each category. Fisher's exact probability test will be performed for statistical comparisons.

Provide Listing of the concomitant medication, concomitant vaccine and non-drug therapy separately.

The above analysis will be based on SS.

7.6 Hypothesis Testing

This study is an exploratory clinical study and will not involve formal hypothesis testing.

7.7 Immunogenicity Evaluation

The following immunogenicity evaluations will be performed separately for EV71 and CA16 neutralizing antibodies in Phase II participants:

7.7.1 Primary Immunization Evaluation

For each group, the seroconversion rate in the overall population, the seroconversion rate in the baseline seronegative population, the 4-fold increase rate in the baseline seropositive population, and the seropositivity rate in the overall population at 30 days after full vaccination will be calculated, respectively. Their 95% confidence intervals will be calculated using the Clopper-Pearson method. The Cochran-Mantel-Haenszel chi-square test (CMH-χ^2) with age group (6–23 months, 24–71 months) as a stratification factor will be used to statistically test differences between groups; pairwise differences between groups will also be statistically tested.

Geometric mean and 95% confidence interval will be computed for the statistical description of the GMT and GMI in the whole population, the baseline seronegative population, and the baseline seropositive population at 30 days after full immunization. Group differences will be compared using ANOVA after logarithmic transformation. Pairwise comparisons will be conducted using two sample t-test after logarithmic transformation.

Reverse cumulative distribution curves of neutralizing antibody titers, before and after the primary vaccination, will be plotted for each group.

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

7.7.2 Immune Persistence Evaluation

The following analysis will be implemented for Phase II participants aged 6 months to 12 years, 6-12 years, 24-71 months, and 6-23 months, respectively.

Calculate the seropositive rates at 6 months after full immunization for each group, and compute their 95% confidence intervals using the Clopper-Pearson method. The CMH chi-square test with age group (6–23 months, 24–71 months) as a stratification factor will be used to statistically test the differences between groups; pairwise differences between groups will also be statistically tested.

Geometric mean and 95% confidence interval will be used for the statistical description of the GMT at 6 months after the full immunization in the baseline seronegative population, the baseline seropositive population, and the overall population. The statistical testing of group differences will be performed using ANOVA after logarithmic transformation. Pairwise comparisons will be conducted using two sample t-test after logarithmic transformation.

Reverse cumulative distribution curves of neutralizing antibody titers at each time point before and after vaccination will be plotted for each group.

Analyses above will be based on IPS.

All immunogenicity data will be tabulated.

7.8 Safety Evaluation

7.8.1 Adverse Event

The following analyses will be based on the Safety Set (SS). The analysis of adverse events for each dose will be based on the safety set for that dose, i.e., SS1 and SS2.

Adverse events and serious adverse events will be coded using MedDRA 26.1 or higher version, classified and statistically analyzed by SOC and PT levels.

This trial primarily conducted statistical analysis on treatment emergent adverse events (TEAE). Adverse events that occurred before the first dose of vaccination will be presented in a listing. Unless otherwise specified, the AEs mentioned hereafter refer to TEAE.

For Phase I and Phase II participants, the number of occurrences, number of participants with events, and incidence of the following types of adverse events will be calculated for the total population and for each group separately, and Fisher's exact test will be used to statistically compare between-group differences for each of the following types of adverse events:

- ✓ Total AEs

- ✓ AEs of abnormal laboratory test
- ✓ Vaccine-related AEs
- ✓ Vaccine-unrelated AEs
- ✓ AEs of different severity grades (Grade 1, 2, 3, 4, 5, ≥Grade 2, and ≥Grade 3)
- ✓ Vaccine-related AEs of different severity grades
- ✓ Vaccine-unrelated AEs of different severity grades
- ✓ AEs at different time points (30 min, 0-7 days, 8-30 days, 0-30 days, more than 30 days)
- ✓ Vaccine-related AEs at different time points
- ✓ Vaccine-unrelated AEs at different time points
- ✓ AEs leading to early withdrawal
- ✓ Vaccine-related AEs leading to early withdrawal

When calculating the incidence of adverse events, if the same participants experience the same adverse event multiple times, it will be counted as one occurrence. When calculating the number of adverse event occurrences, if the same subject experiences the same adverse event multiple times, each occurrence will be counted separately. For the analysis of the severity of adverse events and their relationship to the study vaccine, if the same participant experiences the same adverse event multiple times, the event with the highest severe grade or the strongest relationship to the study vaccine will be selected for statistical analysis.

List all adverse events and adverse events leading to withdrawal, respectively.

7.8.2 Serious Adverse Event

For Phase I and Phase II participants, summarize the number of events, number of participants, and incidence rates of the following types of serious adverse events:

- ➢ Total SAE
- ➢ SAE related to the trial vaccine
- ➢ SAE unrelated to the trial vaccine

Provide listing of serious adverse events.

7.8.3 Laboratory Tests

Analyses will be performed only for Phase I 6-12 year-old and 18-59 year-old participants.

Summarize each laboratory test indicator (including blood routine examination, blood biochemistry and routine urine) at pre- and post-each-dose, as well as the changes before and after each dose of vaccination in each group.

For each group, changes in clinical significance for each laboratory test (including blood routine examination, blood biochemistry, and routine urine) before and after each dose will be presented in the form of cross-tabulations (based on normal reference ranges and the investigator's judgment of clinical relevance).

List all laboratory test results.

In the above analyses, the analysis of laboratory tests for each dose will be based on the safety set for each dose, i.e., SS1 and SS2.

7.9 Handling of Missing Data

In the FAS immunogenicity analysis, missing antibody results at 30 days after full-vaccination will be imputed using the Last Observation Carried Forward (LOCF) method, and the corresponding immunogenicity endpoints will then be derived. For participants with post-vaccination serum results but missing pre-vaccination serum results, the missing pre-vaccination antibody levels will be imputed using the maximum value of pre-vaccination serum antibodies of the same type/antigen across all participants.

This trial will not impute missing data for the endpoints of immunization persistence and safety evaluation.

7.10 Subgroup Analyses

In this study, subgroup analyses are planned for safety in Phase I participants across different age groups (including 18–59 years, 6–12 years, 24–71 months, and 6–23 months), as well as for safety and immunogenicity in Phase II participants across different age groups (including 24–71 months and 6–23 months).

7.11 Multiplicity

No formal hypothesis testing will be performed in this study; therefore, no multiplicity adjustment is considered. Additionally, in the immunogenicity and safety evaluation results, the calculated P-values are only nominal P-values. They are primarily used to describe the strength of the association between

the evaluated endpoint and the treatment group, rather than serving as a basis for formal statistical inference.

VERSION HISTORY

Version	Version Date	Author	Changes of Content
V1.0	2024-09-02	[redacted]	Initial version