Protocol title: A Randomized, Double-Blinded, Active-Controlled Phase III Clinical Trial to Evaluate the Safety and Immunogenicity of Quadrivalent Influenza Vaccine (Split Virion) in Children Aged 6-35 Months

Product name: Influenza Vaccine (Split Virion), Inactivated, Quadrivalent

Sponsor: SINOVAC BIOTECH CO., LTD.

Study Institute:
Hubei Provincial Center for Disease Control and Prevention
Anhui Provincial Center for Disease Control and Prevention

Statistical agency: Beijing Key Tech Statistics Technology Co., Ltd.

Protocol No.: PRO-QINF-3005

Protocol Version Date: Nov. 01, 2023

Version No.: 3.0

Approved by: Zeng Gang

Approver's Signature:

Date of approval: (MM/DD/YY)

北京科兴生物制品有限公司
SINOVAC BIOTECH CO., LTD.

Principal investigator's signatures:

I agree to:

- Be responsible for properly directing the conduct of the clinical study in the region.
- Ensure the study to be conducted in accordance with the trial protocol and standard operating procedures (SOPs).
- Ensure the participants to be fully aware of the investigational product information and other related responsibilities and obligations specified in this trial protocol.
- Ensure no changes to be made to the trial protocol without the review and written approval from the sponsor and Independent Ethics Committee (IEC), unless it is necessary to eliminate the immediate harm to the participant or to comply with the requirements of the registration authority (e.g., administrative aspects of the program).
- Be fully familiar with the proper use of the vaccine as described in the trial protocol and with other information provided by the sponsor, including but not limited to the following: the current investigator's brochure (IB) or the equivalent documents, as well as the IB supplementary (if available).
- Be familiar with and will comply with "Good Clinical Practice (GCP)", "Guidelines for Quality Management of Vaccine Clinical Trials (Trial)" and all the current regulatory requirements.

Name of the principal investigator:

Signature:

Date: (MM/DD/YY)

Protocol Synopsis

Protocol title	A Randomized, Double-Blinded, Active-Controlled Phase III Clinical Trial to Evaluate the Safety and Immunogenicity of Quadrivalent Influenza Vaccine (Split Virion) in Children Aged 6-35 Months
Sponsor	Sinovac Biotech Co., Ltd
Project phase	Phase III
Objective(s)	To evaluate the safety and immunogenicity of Quadrivalent Influenza Vaccines.
Study design	Multi-center, randomized, double-blind, positive-controlled clinical trial
Planned sample size	3300 Participants
Study Population	Children in stable health aged 6-35 months.
Investigation product	**Test vaccines:** Quadrivalent Influenza Vaccine (Split Virion), inactivated, 0.25mL-dose and 0.5mL-dose: -Inactivated influenza strains (H1N1, H3N2, B Victoria and B Yamagata), 7.5μg per strain in 0.25mL-dose and 15μg per strain in 0.5mL-dose -Sodium chloride -Disodium hydrogen phosphate -Sodium dihydrogen phosphate -Water for injection **Control vaccines:** Trivalent Influenza Vaccine (Split Virion), inactivated (Anflu), 0.25mL-dose: -Inactivated influenza strains (H1N1, H3N2, B Victoria), 7.5μg per strain -Sodium chloride -Disodium hydrogen phosphate -Sodium dihydrogen phosphate -Water for injection Trivalent Influenza Vaccine (Split Virion), inactivated (Anflu), 0.25mL-dose: -Inactivated influenza strains (H1N1, H3N2, and B Yamagata), 7.5μg per strain -Sodium chloride -Disodium hydrogen phosphate -Sodium dihydrogen phosphate -Water for injection
Immunization Schedule	Two-dose schedule (0,28 days)

Route of administration	Intramuscularly: anterolateral thigh (participants aged 6-11 months); deltoid region (participants aged 12-35 months)
Challenge schedule, if applicable	None
Safety endpoints	Incidence of adverse reactions 0-28 days after each dose; Incidence of adverse reactions 0-7 days after each dose; Incidence of serious adverse events (SAEs) from the beginning of vaccination to 6 months after the second dose; Incidence of Adverse Events of Special Interest (AESIs) since the beginning of vaccination to 6 months after the second dose.
Immunogenicity endpoints	HI antibody seroconversion rate (SCR), seroprotection rate (SPR), geometric mean titer (GMT) and geometric mean fold increase (GMFI) 28 days after the second dose

Abbreviations

AE	Adverse Event
AESI	Adverse Events of Special Interest
CDC	Center for Disease Control and Prevention
CRF	Case Report Form
EDC	Electronic Data Capture
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
GMI	Geometric Mean Increase
IEC	Independent Ethics Committee
ITT	Intention-to-treat
HI	Hemagglutination inhibition test
NMPA	National Medical Products Administration
PI	Principal Investigator
PPS	Per Protocol Set
SCR	Seroconversion rate
SPR	Seroprotection rate
SOP	Standard Operation Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
SAE	Serious Adverse Event
SS	Safety Set

Summary of the clinical protocol

The Influenza Vaccine (Split Virion), Inactivated, Quadrivalent ("QIV) developed by Sinovac Biotech Co., Ltd. (Sinovac), including two doses of 0.25mL and 0.5mL, is indicated for prevention of influenza caused by influenza viruses of H1N1, H3N2, B Victoria and B Yamagata. The preclinical animal trial results have shown: the vaccine has satisfactory safety and immunogenicity in mice, guinea pigs and rabbits. The 0.5ml-dose QIV was approved for use in individuals aged 3 years and above on June 17, 2020. This Phase III clinical trial plans to evaluate the immunogenicity and safety of the 0.25ml and 0.5ml doses of QIVs developed by Sinovac in children aged 6-35 months. This trial has been approved by the National Medical Products Administration (No.: 2021LB00532). This clinical trial protocol is designed according to the relevant requirements of "Drug Registration Administration" [1], "Good Clinical Practice (GCP)"[2], "Technical Guidelines for Vaccine Clinical Trials"[3] and "Guidelines for Quality Management of Vaccine Clinical Trials (Trial)" of NMPA.

The primary objective of this trial is to evaluate the safety and immunogenicity of 0.25ml-dose and 0.5ml-dose QIV developed by Sinovac in children aged 6-35 months. This trial is a multi-center, randomized, double-blinded, and positive-controlled design. The control vaccine is Anflu®, two0.25mL-dose trivalent influenza virus split vaccines (TIVs, containing a B Victoria lineage or B Yamagata lineage） marketed by Sinovac. This trial will be conducted on children aged 6 to 35 months with the informed consent of their guardians. A total of 3,300 participants are planned to be recruited and randomly assigned to 4 groups in a ratio of 2:2:1:1, namely the 0.25mL-dose QIV group, 0.5ml-dose QIV group, TIV-BV group, and TIV-BY group. All participants receive 2 doses of the test vaccine or control vaccine according to the 0, 28-day immunization schedule.

All participants are observed for immediate reactions for 30 minutes after each dose, solicited systemic and local adverse events within 0-7 days, and unsolicited adverse events within 0-28 days after each dose. Additionally, monitoring of serious adverse events (SAEs) and adverse events of special interest (AESIs) is conducted from the first dose administration to 6 months after the second vaccination to evaluate the safety. Venous blood samples are collected from all participants before immunization and 28 days after the second immunization for hemagglutination inhibition (HI) antibody detection to assess the immunogenicity of the vaccine.

This clinical protocol will be independently undertaken by investigators after obtaining approval from the Ethics Committee. Monitors designated by the sponsor will supervise the standardization of the entire study process to ensure the trial is conducted safely and in compliance with regulations.

Content

1. Introduction

The Influenza Vaccine (Split Virion), Inactivated, Quadrivalent (QIV) developed by Sinovac Biotech Co., Ltd. (Sinovac), including two doses of 0.25mL and 0.5mL, is indicated for prevention of influenza caused by influenza viruses of H1N1, H3N2, B Victoria and B Yamagata. The preclinical animal trial results have shown: the vaccine has satisfactory safety and immunogenicity in mice, guinea pigs and rabbits. The 0.5ml-dose QIV was approved for use in individuals aged 3 years and above on June 17, 2020. This Phase III clinical trial plans to evaluate the immunogenicity and safety of the 0.25ml and 0.5ml doses of QIVs developed by Sinovac in children aged 6-35 months. This trial has been approved by the National Medical Products Administration (No.: 2021LB00532). This clinical trial protocol is designed according to the relevant requirements of "Drug Registration Administration" [1], "Good Clinical Practice (GCP)" [2], "Technical Guidelines for Vaccine Clinical Trials" [3] and "Guidelines for Quality Management of Vaccine Clinical Trials (Trial)" of NMPA.

2. Background and rationale

Influenza (flu) is an acute respiratory infectious disease caused by influenza viruses, which can cause outbreaks and epidemics worldwide. The disease burden imposed by influenza varies across different regions and populations. Globally, approximately 5%-10% of adults and 20%-30% of children suffer from seasonal influenza each year, leading to 3 to 5 million severe cases and 250,000 to 500,000 deaths annually, posing a serious threat to public health. The main clinical manifestations include fever, headache, myalgia, fatigue, rhinitis, sore throat, and cough. Over the past three centuries, influenza has been one of the major infectious diseases. At present, the transmission routes of influenza viruses and the continuous mutation of the virus remain the main obstacles to the prevention and control of influenza.

2.1 Etiology

Influenza viruses belong to the Orthomyxoviridae family, and are single-stranded, negative-sense, segmented RNA viruses. Based on their nucleoprotein and matrix protein, influenza viruses are classified into four types: A, B, C, and D. Influenza A viruses can be further divided into multiple subtypes according to the protein structure and genetic characteristics of hemagglutinin (HA) and neuraminidase (NA) on the viral surface. Currently, 18 HA subtypes (H1-H18) and 11 NA subtypes (N1-N11) have been identified [5]. In addition to infecting humans, influenza A viruses are widely present in animals, such as poultry, pigs, horses, seals, whales, and minks. Influenza B viruses are divided into Victoria lineage and Yamagata lineage, which can circulate among the human population, and recent data have shown that seals can also be infected. Influenza C viruses infect humans, dogs, and pigs, and only cause sporadic cases of upper respiratory tract infections [6]. Influenza D viruses mainly infect pigs, cattle, etc., and no human infections have been found so far [7-9]. At present, the viruses causing seasonal influenza epidemics are H1N1 and H3N2 subtypes of influenza A, as well as Victoria and Yamagata lineages of influenza B.

2.2 Epidemiology

Patients with influenza and asymptomatic carriers are the main sources of infection for seasonal influenza. The virus is primarily transmitted through droplets from their respiratory secretions, and can also be spread via direct or indirect contact with the mucous membranes of the mouth, nose, eyes, etc. The common incubation period is 1-4 days (with an average of 2 days), and infectivity persists from the end of the incubation period to the acute phase of the illness. Generally, infected individuals can shed the virus 24-48 hours before the onset of clinical symptoms; the amount of virus shedding increases significantly 0.5-1 day after infection and reaches a peak within 24 hours of the onset of illness [10]. Adults and older children usually continue to shed the virus for 3-8 days (average 5 days). Younger children shed the same amount of virus as adults when they fall ill, but the decrease in virus shedding is slower and the duration of shedding is longer. Compared with adults, prolonged virus shedding (1-3 weeks) is common in children.

In temperate regions, influenza exhibits seasonal outbreaks and high incidence during winter and spring each year. In 2013, a study on the seasonality of influenza in different regions of China [11] showed that the annual periodicity of influenza A in China strengthens with increasing latitude, presenting diverse spatial patterns and seasonal characteristics: in northern provinces north of 33°N, it shows a winter epidemic pattern with a single annual peak in January to February; in the southernmost provinces south of 27°N, there is a single annual peak in April to June; in the mid-latitude regions, there are two periodic peaks in January to February and June to August. While influenza B shows a single winter peak in most areas of China. In 2018, a systematic analysis study was conducted on the epidemiological characteristics of influenza B in China from 2005 to 2016 [12]. Overall, the epidemic intensity of influenza B in China is lower than that of influenza A; however, in some regions and years, the epidemic intensity of influenza B is higher than that of influenza A. Additionally, the B/Yamagata lineage and B/Victoria lineage alternately dominate, with epidemics mainly occurring in winter and spring, and the epidemic intensity of different lineages varies across years.

During annual influenza seasons, the incidence of influenza in children is approximately 20-30%, and in some high-prevalence seasons, the annual infection rate in children can reach around 50% [13, 14]. A review study on influenza incidence (including both symptomatic and asymptomatic infections) [15] indicated that the influenza incidence rate in children under 18 years old is approximately 22.5% (95% CI: 9.0-46.0%), while that in adults is about 10.7% (95% CI: 4.5-23.2%). A study on influenza infection and incidence rates in Beijing during the 2017-2018 season showed that the 0-4 years age group and 5-14 years age group had the highest incidence rates, at 33.0% (95% CI: 26.4-43.1%) and 21.7% (95% CI: 17.4-28.4%), respectively [16]. A study in Suzhou reported that the consultation rate for influenza-like illness (ILI) caused by confirmed influenza in children under 5 years old during the 2011-2017 seasons was 6.4 per 100 person-years, with the highest rate in the 2011-2012 season (20.5 per 100 person-years) and the lowest in the 2012-2013 season (2.4 per 100 person-years) [17].

Children under 5 years old are at a high risk of developing severe illness after influenza infection. A systematic review and modeling study on the burden of influenza-related respiratory infections in children under 5 years old worldwide [18] indicated that in 2018, approximately 109.5 million (uncertainty range [UR]: 63.1-190.6 million) children under 5 years old globally were infected with influenza viruses, 10.1 million (UR: 6.8-15.1 million) developed influenza-related acute lower

respiratory infections (ALRI), 870,000 (UR: 543,000-1.415 million) were hospitalized due to influenza-related ALRI, 15,300 (UR: 5,800-43,800) in-hospital deaths occurred among influenza-related ALRI, and 34,800 (UR: 13,200-97,200) deaths were attributed to influenza-related ALRI cases. A population-based study in Jingzhou City, Hubei Province, found that 69% of hospitalized cases with severe acute respiratory infection (SARI) caused by influenza were children under 5 years old, with the influenza-related SARI hospitalization rate in this age group reaching 2,021-2,349 per 100,000 person-years, and the highest rate among infants aged 6-11 months (3,603-3,805 per 100,000 person-years) [19]. A study in Suzhou on the confirmed influenza-related SARI hospitalization rate in children under 5 years old during the 2011-2016 seasons [20] showed that the rate ranged from 4 cases per 1,000 people (95% CI: 2-5 cases per 1,000 people) in the 2012-2013 season to 16 cases per 1,000 people (95% CI: 14-19 cases per 1,000 people) in the 2011-2012 season. Influenza infection in children can lead to death. Children with underlying diseases have a significantly higher risk of death than healthy children, but nearly half of the fatal cases occur in healthy children [21]. A modeling study on global influenza-related deaths estimated that approximately 9,243-105,690 children under 5 years old die annually from influenza-related respiratory diseases in the 92 included countries [22].

2.3 Research, Development of Quadrivalent Influenza Vaccines

Before 1976, influenza vaccines in the United States contained only two virus strains. The components of seasonal influenza vaccines included three types of virus strains recommended by the World Health Organization (WHO) or the U.S. Centers for Disease Control and Prevention (CDC) since 1976. Since the 1980s, the global epidemic has been characterized by the concurrent circulation and co-transmission of two B-type virus lineages, Yamagata and Victoria, with poor cross-protection between them. Therefore, in recent years, most domestic and foreign vaccine manufacturers have recognized the necessity of developing quadrivalent influenza vaccines. Currently, some manufacturers have obtained approval for their quadrivalent influenza vaccines to be marketed, some are conducting clinical studies on quadrivalent influenza vaccines.

In December 2012, U.S. FDA approved GlaxoSmithKline (GSK)'s QIV (Fluarix Quadrivalent), which was also the first QIV in history, available in pre-filled syringes. On August 16, 2013, GSK's second quadrivalent influenza vaccine, FluLaval Quadrivalent, was also approved by the U.S. FDA for active immunization in individuals aged 6 months and older. Both vaccines are used to prevent influenza caused by seasonal influenza A and B strains. On June 10, 2013, Sanofi Pasteur announced that the U.S. FDA had approved its QIV (Fluzone® Quadrivalent) for active immunization in individuals aged 6 months and older to prevent influenza caused by seasonal influenza A strains and B strains. In August 2016, the U.S. FDA approved Seqirus' quadrivalent influenza vaccine Afluria® Quadrivalent for the first time, for use in adults aged 18 years and older to prevent influenza caused by seasonal influenza A and B strains and approved the vaccine for use in individuals aged 6 months and older in October 2018.

For the 2022-2023 influenza season, the U.S. Center for Disease Control and Prevention (CDC) recommends quadrivalent influenza vaccines for children aged 6-35 months as shown in Table 1.

Table 1 Quadrivalent Influenza Vaccines Recommended by the U.S. CDC for Children Aged 6-35 Months in the 2022-23 Influenza Season

Trade Name (Manufacturer)	Doses
Afluria® Quadrivalent (Seqirus)	0.25 mL (7.5μg) and 0.5ml（15μg）
Fluarix® Quadrivalent (GlaxoSmithKline)	0.5 mL (15μg)
FluLaval® Quadrivalent (GlaxoSmithKline)	0.5 mL (15μg)
Fluzone® Quadrivalent (Sanofi Pasteur)	0.25 mL (7.5μg) or 0.5 mL (15μg)
Flucelvax® Quadrivalent (Seqirus), cell substrate	0.5 mL (15μg)

Since 2013, domestic vaccine manufacturers have recognized the necessity of developing quadrivalent influenza vaccines. At present, several domestic manufactures have launched quadrivalent influenza vaccines on the market, but only three of them are intended for children aged 6-35 months. These include the quadrivalent influenza vaccine from Hualan Biological Engineering, Inc., Shanghai Institute of Biological Products Co., Ltd. and Shenzhen Sanofi Pasteur Biological Products Co., Ltd.

2.4 Study rationale

Immunization Schedule: The target population of this clinical trial is children aged 6-35 months, with an immunization schedule of 2 doses (28 days apart), consistent with the approved trivalent or quadrivalent influenza vaccines currently on the market.

Vaccination Dose: The vaccination doses in this clinical trial include the 0.25ml dose (containing 7.5μg of hemagglutinin for each strain) and the 0.5ml dose (containing 15μg of hemagglutinin for each strain). Among them, the 0.25ml influenza vaccine has been used in the 6-35 months age group for over 30 years. Initially, half-dose antigen influenza vaccines were developed to reduce the risk of fever convulsions that might be caused by whole-virus influenza vaccines [23]. However, several recent studies have shown that children under 3 years old can receive full-dose vaccines to enhance immunogenicity without increasing the severity or incidence of fever or other adverse reactions [24-26]. In the United States, the 0.5ml dose of QIV was first approved for use in children aged 6-35 months in 2016. Currently, multiple domestic manufacturers are also conducting clinical trials on both 0.25ml and 0.5ml doses vaccines in this population.

Control Selection: In this clinical trial, two 0.25ml trivalent influenza vaccines (TIVs, Anflu®) marketed by Sinovac were selected as the control vaccines. Anflu® has been marketed and used in China for 20 years, with previous study results showing good protective efficacy, immunogenicity, and safety. The immunogenicity results would be comparable due to the same strains and manufacturing process between test and control vaccines. Additionally, relevant studies have shown that some indicators of immune responses to the two B serotypes in quadrivalent influenza vaccines may be lower than those in trivalent influenza vaccines. For example, in a clinical study of quadrivalent influenza vaccines in the 6-35 months age group, the geometric mean titers (GMT) of HI antibodies against the BV serotype at 28 days after full immunization were 64.0 in the 0.25ml quadrivalent influenza vaccine group and 91.3 in the trivalent influenza vaccine (containing BV) group; the GMTs of HI antibodies against the BY serotype were 96.6 in the 0.25ml quadrivalent influenza vaccine group and 106 in the trivalent influenza vaccine (containing BY) group [27].

Therefore, two trivalent influenza vaccines were selected as controls in this study. Furthermore, since domestic marketed influenza vaccines currently lack data on protective efficacy, Sinovac will conduct post-marketing protective efficacy studies in a timely manner to accumulate data on the effectiveness of seasonal influenza vaccines in China.

3. Preclinical study results

See in Investigator's Brochure (IB).

4. Preliminary Clinical Studies

See in Investigator's Brochure (IB).

5. Product Characteristics

5.1 Preparation Process and Formulation of the Test Vaccine

The quadrivalent influenza virus split vaccine is prepared by inoculating the influenza A and B virus strains recommended by the World Health Organization (WHO) for the 2023-2024 Northern Hemisphere influenza season and approved by the national food and drug regulatory authority into chicken embryos, respectively. The vaccine is manufactured through virus culture, harvest of virus liquid, virus inactivation, purification, and splitting. The test vaccine is a clear liquid, packaged in pre-filled syringes, with doses of 0.25ml or 0.5ml per syringe.

The strains contained in the test vaccine (0.25ml or 0.5ml) are the strains recommended for the 2023-2024 Northern Hemisphere influenza season, as follows:

A/Victoria/4897/2022 (H1N1) pdm09-like virus ……7.5μg or 15μg hemagglutinin
A/Darwin/9/2021 (H3N2)-like virus ……7.5μg or 15μg hemagglutinin
B/Austria/1359417/2021 (B/Victoria lineage) ……7.5μg or 15μg hemagglutinin
B/Phuket/3073/2013 (B/Yamagata lineage) ……7.5μg or 15μg hemagglutinin

Inactive ingredients include sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, and water for injection. The test vaccine is prepared by Sinovac Biotech Co., Ltd., and has been inspected by the National Institutes for Food and Drug Control, with all test results meeting the requirements of the Manufacturing and Inspection Procedures for Quadrivalent Influenza Virus Split Vaccine (draft) or Manufacturing and Inspection Procedures for Quadrivalent Influenza Virus Split Vaccine (approval number: 2020S00335).

5.2 Stability of the Vaccine

When stored at 2-8°C for 12 months, all test results of the test vaccine meet the quality standards.
When stored at 25°C for 14 days, all test results meet the quality standards; when stored at 25°C for 21 days, the hemagglutinin content fails to meet the quality standards.

When stored at 37°C for 3 days, all test results meet the quality standards; when stored at 37°C for 7 days, the hemagglutinin content fails to meet the quality standards.

Based on the stability test results at 2-8°C and with reference to the stability results at 25°C and 37°C, the validity period of the test vaccine is set as 1 year.

5.3 Control Vaccines

The control vaccines (Anflu) are trivalent influenza virus split vaccines prepared with the strains recommended by WHO for the 2023-2024 Northern Hemisphere influenza season. Control Vaccine 1 is the marketed Anflu for the 2023-2024 influenza season, containing 7.5μg each of H1N1, H3N2, and BV antigens. Control Vaccine 2 contains 7.5μg each of H1N1, H3N2, and BY antigens. The sources of antigen strains for each type in the control vaccines are consistent with those corresponding to the quadrivalent influenza vaccine. The control vaccines are prepared by Sinovac Biotech Co., Ltd., packaged in pre-filled syringes, with a dose of 0.25ml per syringe, and have been inspected by the National Institutes for Food and Drug Control, meeting the requirements of the Manufacturing and Inspection Procedures for Influenza Virus Split Vaccine.

5.4 Storage and Transportation of the Vaccine

The vaccine should be stored and transported at 2-8°C in the dark; freezing is strictly prohibited.

5.5 Vaccination Route and Schedule

Eligible participants will receive 2 doses of the 0.25ml or 0.5ml test vaccine or control vaccine according to the 0, 28-day schedule. Participants aged 6-11 months (before their first birthday) will receive intramuscular injection in the anterolateral thigh, and those aged 12-35 months will receive intramuscular injection in the deltoid muscle of the upper arm.

5.6 Information on the Test Vaccine

Information on the vaccine used in the clinical trial is shown in the table below:

Table 2 Information on Vaccines Used in Clinical Trial

Group	Vaccine Name	Manufacturer	Dosage	Batch No.	Expiry Date
Test Group 1	Quadrivalent Influenza Virus Split Vaccine	Sinovac	7.5μg per strain / 0.25ml	LA20230502T	2024.05.23
Test Group 2	Quadrivalent Influenza Virus Split Vaccine	Sinovac	15μg per strain / 0.5ml	LA202305002	2024.05.21
Control Group 1	Anflu (containing B Victoria)	Sinovac	7.5μg per strain / 0.25ml	CA202305001	2024.05.21
Control Group 2	Anflu (containing B Yamagata)	Sinovac	7.5μg per strain / 0.25ml	CA20230507T	2024.05.23

6. Study Objectives

6.1 Primary objective

- To evaluate the non-inferiority of immunogenicity in participants aged 6-35 months after 2 doses of 0.5mL-dose QIV compared with two TIVs (including BV or BY) for the shared strains, in terms of seroconversion rate (SCR) and Geometric mean titer (GMT) for HI antibody;

- To evaluate the non-inferiority of immunogenicity in participants aged 6-35 months after 2 doses of 0.25mL-dosen QIV compared with two TIVs (including BV or BY) for the shared strains, in terms of SCR and GMT for HI antibody;

- To evaluate the absolute criteria of HI antibody SCR and seroprotection rate (SPR) for each strain in participants aged 6-35 months after two doses of 0.25mL-dose or 0.5mL-dose QIVs.

6.2 Secondary objective

- To evaluate the non-inferiority of immunogenicity in participants aged 6-35 months after 2 doses of 0.5mL-dose QIV compared with 0.25mL-dose QIV for each strain, in terms of seroconversion rate and GMT of HI antibody;

- To evaluate the superiority of immunogenicity in participants aged 6-35 months after 2 doses of 0.25mL-dose or 0.5mL-dose QIVs compared with two TIVs (including BV or BY) for the unique strain, in terms of seroconversion rate and GMT of HI antibody;

- To evaluate the immunogenicity in participants aged 6-35 months after vaccination of 2 doses of 0.25mL-dose or 0.5mL-dose QIVs;

- To evaluate the safety of 0.25mL-dose or 0.5mL-dose QIVs in participants aged 6-35 months.

7. Study design

7.1 Design

7.1.1 Overall study design

A multi-centered, randomized, blinded (double-blinded), and controlled trial design.

7.1.2 Sample size calculation

This trial requires the test group: ① the non-inferiority comparison with the control groups is established and the next statistical test is carried out; ② when the absolute criteria of SCR and SPR are achieved, the trial can be considered as successful and a positive conclusion can be drawn for the test group. In this trial, when evaluating between the test group 1 (0.25-mL QIV) and test group 2 (0.5mL-QIV), the strategy of sequential test is adopted. First, test group 2 is evaluated. If the positive conclusion of test group 2 is established, test group 1 is further evaluated. Therefore, class I error correction is no longer carried out. The sample size of phase III clinical trials is estimated first by non-inferiority design, namely the QIV group is non-inferior to the TIV group. The primary endpoints of this study include two indicators: SCR and GMT. Only when the SCR and GMT of four serotypes in test group are non-inferior to the control vaccine, the investigational vaccine is considered to be non-inferior to the control vaccine. The total power is 80%, the type II

error is 0.2. Based on the different sample size requirements of the two indicators, the type II error is allocated, with 0.16 for seroconversion rate comparison and 0.04 for GMT comparison.

(1) Calculation of sample size with the SCR as the indicator:
According to the results of the previous Anflu phase IV clinical trial and other literature, the SCR of the types contained in the vaccine 28 days after vaccination is over 65%, which is used as the reference proportion to estimate the sample size. The test level is taken as one-sided α=0.025, the power of non-inferiority comparison for the seroconversion rate of a serotype is taken as 1-β1/4=1-0.16/4=96%, and the non-inferiority threshold Δ is taken as -10%. When comparing the non-inferiority of BY or BV strain, NT:NC=2:1, and PASS software is used to calculate the sample size, the test group needs 914 samples and the control group needs 457 samples. When the non-inferiority test of H1N1 or H3N2 strain is carried out, two control groups are pooled. The sample size ratio of the test group and the pooled control group is 1:1, and the test group and the pooled control group need 625 participants.

(2) Calculation of sample size with the GMT as the indicator:
Using the antibody GMT 28 days after vaccination as the outcome measure, the non-inferiority margin is ≥2/3 of the 95%CI lower limit of GMT ratio between the test group and the control group, and the non-inferiority margin after logarithmic conversion is Δ=-0.176. The test level is taken as one-sided α=0.025, and the power of a certain serotype GMT for non-inferiority comparison is taken as 1-β2/4=1-0.04/4=99%, and the standard deviation after logarithmic transformation is taken as σ =0.7 with reference to the previous research results. When comparing the non-inferiority of BY or BV, NT:NC=2:1, and PASS software is used to calculate the sample size, the test group needs 874 samples and the control group needs 437 samples. When the non-inferiority test of H1N1 or H3N2 type is carried out, two control groups are pooled. The sample size ratio of the test group and the pooled control group is 1:1, and the test group and the pooled control group need 582 participants each.

When the SCR and GMT of the test group reach the hypothesis of non-inferiority, the absolute criteria of the SCR and seroprotection rate (SPR) of the test group are further hypothesized for test. The trial requires that the SCR and SPR of the four strains of immune antibodies reach the absolute criteria, and the total power of the trial is 80%, so the power of each test is 1- (0.2/8)=0.975.

(3) Calculation of sample size with the absolute criteria of SCR:
Assuming that the SCR after vaccination is 65%, the test level is one-sided α=0.025, the lower limit of the confidence interval of the seroconversion rate should be greater than 30%, and the power of each test is 97.5%. The sample size is calculated by PASS software, and the test group needs 28 samples.

(4) Calculation of sample size with the absolute criteria of SPR:
Assuming that the seroconversion rate after vaccination is 75%, the test level is one-sided α=0.025, the lower limit of the confidence interval of the SPR should be greater than 60%, and the power of each test is 97.5%. The sample size is calculated by PASS software, and the test group needs 146 samples.

In consideration of the above the maximum sample size and the dropout rate of about 17%, the sample size of each test group is 1,100, and the sample size of each control group is 550, with a total sample size of 3,300.

7.2 Study endpoint

Co-primary endpoint and evaluation criteria:

(1) The HI antibody GMTs and SCRs for each of the four strains in QIV groups were inferior to those of control groups at 28 days after vaccination in total populations. The non-inferiority criteria are as follows:

- **GMTs:** Non-inferiority of GMTs was demonstrated if the lower limit of the two-sided 95% confidence interval (CI) for adjusted GMT ratio (test group/control group) ≥ 2/3 for each of the four strains.
- **SCRs:** The percentages of participants with either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40, or a pre-vaccination titer ≥ 10 and a ≥ 4-fold increase in post-vaccination titer at 28 days after vaccination. Non-inferiority for SCR was demonstrated if the lower limit of the two-sided 95% CI for SCR difference (test group-control group) was ≥-10% for each of the 4 strains.

(2) The SCRs and SPRs should meet the absolute criteria in total populations.

- Absolute criteria of SCR and SPR: the lower limit of the two-sided 95%CI for SCR was ≥30%; the lower limit of the two-sided 95%CI for SPR was ≥60%.

Second endpoint and evaluation criteria:

➢ **Immunogenicity endpoint**

(1) The HI antibody GMTs and SCRs for the unique B strain in QIV groups were superior to those of control groups at 28 days after vaccination in total populations. The criteria for superiority were as follows:

- The lower limit of the two-sided 95%CI for adjusted GMT ratio (test group/control group) ≥1.5;
- The lower limit of the two-side 95% CI for SCR difference (test group-control group) was ≥ 10%.

(2) The HI antibody GMTs and SCRs for each of the four strains in 0.5mL-dose QIV group were inferior to those of 0.25-mL-dose QIV at 28 days after vaccination in total populations. The non-inferiority criteria were consistent with the co-primary endpoints.

(3) The HI antibody GMIs of each strain at 28 days after second vaccination in total population;

(4) The HI antibody SCRs, SPRs, GMTs and geometric mean fold increasing (GMFIs) of 0.5mL-dose QIV and 0.25mL-dose QIV in susceptible population (HI antibody titers ＜1:10). The non-inferiority, superiority and absolute criteria refer to the total population.

➢ **Safety endpoint**

(1) Incidence of adverse reactions in 0-28 days after each dose of QIVs;
(2) Incidence of adverse reactions in 0-7 days after each dose of QIVs;
(3) Incidence of SAE from the beginning of the first dose to 6 months after second dose;
(4) Incidence of AESI from the beginning of first dose to 6 months after second dose.

7.3 Estimand

7.3.1 Primary Estimand

Table 3 Primary Estimand

	Primary Estimand
Population	The target population involves all participants aged 6 to 35 months who have completed two doses of vaccination.
Variable	(1) Whether the H1N1 and H3N2 HI antibodies are seroconverted in the test group (0.5mL or 0.25mL) and the pooled TIV group 28 days after the second dose. (2) Whether the BV HI antibody is seroconverted in the test group (0.5mL or 0.25mL) and the TIV-BV groups 28 days after the second dose. (3) Whether the BY HI antibody is seroconverted in the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose. (4) H1N1 and H3N2 HI antibody titers of the test group (0.5mL or 0.25mL) and the pooled TIV group 28 days after the second dose. (5) BV HI antibody titers of the test group (0.5mL or 0.25mL) and the TIV-BV groups 28 days after the second dose. (6) BY HI antibody titers of the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose. (7) Whether the H1N1, H3N2, BV, and BY HI antibody titer reach the protective antibody level in the test group (0.5mL or 0.25mL) 28 days after the second dose.
Treatments	Subjects received two doses of the test vaccine or the control vaccine according to the 0,28-day schedule.
Intercurrent Events and Treatment Strategies	The definition is shown in Table 4.
Population-level Summary	(1) Difference between the SCRs of H1N1 and H3N2 HI antibodies of the test group (0.5mL or 0.25mL) and the pooled TIV group 28 days after the second dose. (2) Difference between the SCRs of BV serum HI antibodies of the test group (0.5mL or 0.25mL) and the TIV-BV group 28 days after the second dose. (3) Difference between the SCRs of BY HI antibodies of the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose vaccination. (4) GMT ratios of H1N1 and H3N2 HI antibodies of the test group (0.5mL or 0.25mL) and the pooled TIV group 28 days after the second dose (5) GMT ratios of BV serum HI antibodies of the test group (0.5mL or 0.25mL) and the trivalent control group (including BV) after 28 days of full-course vaccination. (6) GMT ratios of BY serum HI antibodies of the test group (0.5mL or 0.25mL) and the trivalent control group (including BY) after 28 days of full-course vaccination. (7) Seroprotection rates (SPRs) of H1N1, H3N2, BV, and BY HI antibody in the test group (0.5mL or 0.25mL) after 28 days after the second dose.

Table 4 Intercurrent Events and Treatment Strategies of Primary Estimand

Intercurrent Event	Treatment Strategy	Remarks
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

7.3.2 Secondary Estimand 1

Table 5 Secondary Estimand 1

	Secondary Estimand 1
Population	The target population involves all participants aged 6 to 35 months who have completed two doses of vaccination.
Variable	(1) Whether the H1N1, H3N2, BV, and BY HI antibodies are seroconverted in both the test group 2 (0.5mL) and the test group 1 (0.25mL) 28 days after the second dose. (2) H1N1, H3N2, BV, and BY serum HI antibody titers of the test group 2 (0.5mL) and the test group 1 (0.25mL) 28 days after the second dose.
Treatments	As the same as the primary estimand
Intercurrent Events and Treatment Strategies	As the same as the primary estimand
Population-level Summary	(1) Difference between the SCRs of H1N1, H3N2, BV, and BY serum HI antibodies of the test group (0.5mL) and the test group (0.25mL) 28 days after the second dose. (2) GMT ratios of H1N1, H3N2, BV, and BY serum HI antibodies between the test group (0.5mL) and the test group (0.25mL) 28 days after the second dose.

7.3.3 Secondary Estimand 2

Table 6 Secondary Estimand 2

	Secondary Estimand 2
Population	The target population involves all participants aged 6 to 35 months who have completed two doses of vaccination.
Variable	(1) Whether the BV HI antibody is seroconverted in the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose. (2) Whether the BY HI antibody is seroconverted in the test group (0.5mL or 0.25mL) and

	Secondary Estimand 2
	the TIV-BV group 28 days after the second dose. (3) BV HI antibody titers of the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose. (4) BY HI antibody titers of the test group (0.5mL or 0.25mL) and the TIV-BV group 28 days after the second dose.
Treatments	As the same as the primary estimand
Intercurrent Events and Treatment Strategies	As the same as the primary estimand
Population-level Summary	(1) Difference between the SCRs of BV HI antibody of the test group (0.5mL or 0.25mL) and TIV-BY group 28 days after the second dose. (2) Difference between the SCRs of BY serum HI antibody of the test group (0.5mL or 0.25mL) and TIV-BV 28 days after the second dose. (3) GMT ratios of BV HI antibodies of the test group (0.5mL or 0.25mL) and the TIV-BY group 28 days after the second dose. (4) GMT ratios of BY HI antibodies of the test group (0.5mL or 0.25mL) and the TIV-BV group 28 days after the second dose.

7.3.4 Secondary Estimand 3

Table 7 Secondary Estimand 3

	Secondary Estimand 3
Population	The target population involves the subjects (susceptible population) with the HI antibody titer <1:10 before immunization, aged 6 to 35 months, who have completed the full-course vaccination of two doses.
Variable	As the same as the primary estimand
Treatments	As the same as the primary estimand
Intercurrent Events and Treatment Strategies	As the same as the primary estimand
Population-level Summary	As the same as the primary estimand

7.4 Study plan

This trial is a multi-center, randomized, double-blinded, and positive-controlled design. The control vaccine is Anflu®, two 0.25mL-dose trivalent influenza virus split vaccines (TIVs, containing a B Victoria lineage or B Yamagata lineage） marketed by Sinovac. This trial will be conducted on children aged 6 to 35 months with the informed consent of their guardians. A total of 3,300 participants are planned to be recruited and randomly assigned to 4 groups in a ratio of 2:2:1:1, namely the 0.25mL-dose QIV group, 0.5ml-dose QIV group, TIV-BV group, and TIV-BY group. All participants receive 2 doses of the test vaccine or control vaccine according to the 0, 28-day immunization schedule.

All participants are observed for immediate reactions for 30 minutes after each dose, solicited systemic and local adverse events within 0-7 days, and unsolicited adverse events within 0-28 days after each dose. Additionally, monitoring of serious adverse events (SAEs) and adverse events of special interest (AESIs) is conducted from the first dose administration to 6 months after the second vaccination to evaluate the safety. Venous blood samples are collected from all participants before immunization and 28 days after the second immunization for hemagglutination inhibition (HI) antibody detection to assess the immunogenicity of the vaccine.

Table 8 Study design

Group	Vaccines	Sample size	Immunization schedule (days)	Blood sample collection (days)
Test group 1	Influenza Vaccine (Split Virion), Inactivated, Quadrivalent (0.25mL)	1100	0,28	0,56
Test group 2	Influenza Vaccine (Split Virion), Inactivated, Quadrivalent (0.5mL)	1100	0,28	0,56
Control group 1	Influenza Vaccine (Split Virion), Inactivated, Anflu (0.25mL, including BV)	550	0,28	0,56
Control group 2	Influenza Vaccine (Split Virion), Inactivated, Anflu (0.25mL, including BY)	550	0,28	0,56
Total		3300		

7.5 Randomization and blinding

7.5.1 Randomization

This clinical trial is a randomized, blinded, positive-controlled design. Then, random blind code will be generated by a third-party randomization statistician independent of the project using the SAS statistical software (version 9.4) according to the preset block lengths, and the range of random numbers is specified in the "Randomization protocol". Vaccines are randomized and blinded according to random numbers. The blind code of investigational vaccines is the "List of Correspondence Between Investigational Vaccine Codes and Vaccine Groups", will be stored by the randomization statistician.

At the same time, the randomization statistician uses SAS statistical software to generate the backup vaccine blind code. When the investigational vaccine is damaged, the vaccinator should report to the on-site technical head and the Principal Investigator to start the spare vaccine program. The numbers of backup vaccines are specified in the "Randomization Scheme". All investigational vaccines need to be re-labeled with blind labels, and the label format is detailed in "6.7 Vaccine Packaging". After enrollment, each subject will receive the blinded vaccine corresponding to their study number.

7.5.2 Blinding

This clinical trial adopts a double-blind design. Randomization statisticians and other blinding staff who did not participate in the trial performed vaccine blinding, that is, pasting the printed label on the designated position of each vaccine according to the blind code. The randomization statistician

supervises the blinding of vaccines, and guides the blinding operators to label according to the blind code. After completion of blinding, the blind code should be sealed by the randomization statistician. The entire process of blinding must be recorded. The blinding personnel must not be involved in other relevant work in this clinical trial or reveal the blind code to anyone who is involved in this clinical trial.

Considering 0.25mL and 0.5mL investigational vaccines were used for this clinical trial, the following measures were taken to maintain the blind status of the study process:

(1) Investigators engaged in vaccination and vaccine administrators must sign confidentiality agreements, and cannot participate in other clinical work involving research information of participants;

(2) After verifying that the information of the participants is correct, the vaccine administrator should take out the vaccine with the corresponding number and submit it to the vaccinators in this group. The vaccinators must check the information of the participants again and carry out vaccination after confirmation;

(3) Each participant must be vaccinated individually, meaning that there are only the vaccination personnel, the subject, and his/her accompanying person in the vaccination room during each vaccination.

7.5.3 Emergency Unblinding

Based on the safety of participants and other special reasons, the treatment group information of individual or some participants can be obtained in emergency situations in accordance with pre-established standard operating procedures (SOPs). For both expected and unexpected serious adverse events, emergency unblinding shall only be conducted when researchers must know the group information due to an emergency involving the subject (e.g., requiring rescue). If knowledge of the group information is not necessary for handling the emergency, emergency unblinding is not required.

Once emergency unblinding occurs, the time, reason, and executor of the emergency unblinding must be recorded promptly. Meanwhile, the monitor shall be notified as soon as possible, and a safety event report shall be submitted to the Ethics Committee. After the trial is completed, the number, reasons, scope, and timing of emergency unblinding shall be described and analyzed, serving as a reference for the evaluation of immunogenicity and safety.

7.5.4 SUSAR Unblinding

In the event of a suspected unexpected serious adverse reaction (SUSAR), the sponsor needs to unblind the individual case report to facilitate judging the correlation between the serious adverse event and the trial vaccine and determining whether rapid reporting is required.

This clinical trial adopts a blinded design. When a situation requiring SUSAR unblinding occurs, the non-blinded pharmacovigilance personnel shall apply to the randomization statistician via email for the blind code information of the corresponding subject. After unblinding, if the vaccine involved is the trial vaccine, it shall be submitted in accordance with the rapid reporting procedures and requirements; the reporting method is specified in the submission process from the sponsor to the regulatory authorities in "9.7.6 Reporting of Serious Adverse Events". This unblinding process

shall be conducted independently, and the SUSAR unblinding of individual case reports will not affect the implementation of the clinical trial or the analysis of the final results.

7.5.5 Unblinding Regulations

Unblinding of this clinical trial will be conducted after obtaining the serum test results of all participants at 28 days after the second dose, which will be jointly implemented by the sponsor, principal investigator, and statistical party. After unblinding, personnel responsible for subject observation, result judgment, and data verification will remain blinded until the final database is locked.

7.6 Clinical Trial Flowchart

The flowchart of this clinical trial is shown in Figure 1.

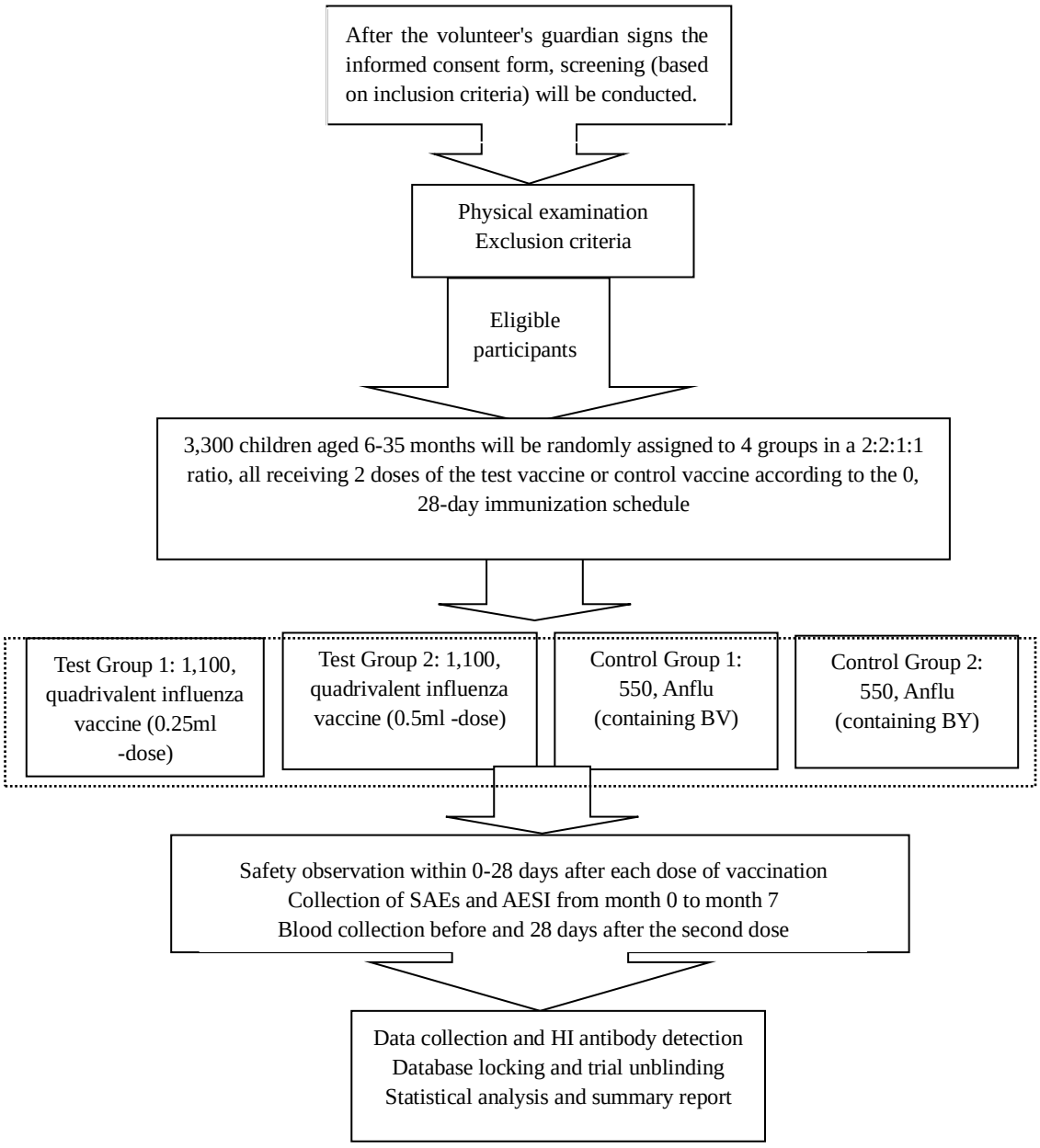

Figure 1 Flowchart of Phase III Clinical Trial

7.7 Study duration

7.7.1 Duration of the Study

Table 9 Study period

Items	Period
Trial preparation (site selection, protocol and case report form design; ethical review; site registration; application to the Ethics Committee for Genetic Resources; database set-up; subject recruitment, etc.)	3 months
On site operation +Data collection +Antibody detection	10 months
Statistical analysis	1 month
Finish clinical study report	1 month
Total	15 months

7.7.2 Duration of Subject Participants

The expected period is no less than 7 months for each participant.

7.8 Suspension and early termination

The Criteria of the trial suspension:

(1) Occurrence of one or more the Grade 4 vaccine-related adverse events (local and systemic), or fatal or life-threatening SUSARs;

(2) Occurrence of Grade 3 or above adverse events in ＞15% participants or more, including local reactions, systemic reactions and vital signs.

The Criteria of early termination of the trials:

(1) The sponsor requests termination of the trial and provides the reasons;

(2) EC requests termination of the trial and provides the reasons;

(3) The requests termination of the trial and provides the reasons; and

(4) After the clinical trial is suspended, the investigator and the sponsor shall discuss whether to terminate the trial or not.

When the occurrence of adverse events meets the suspension criteria, the trial will be suspended first through determination of investigators and the sponsor. In case of disagreements between investigator and the sponsor, the investigators' judgment shall prevail. Secondly, the suspension shall be reported to the national drug regulatory department and EC, as well as be informed to the participants. On the basis of analyzing the relevance of the adverse events as well as the severity and safety risks, an investigation report will be formed. Relevant parties to jointly decide whether to continue the clinical trial.

7.9 Protocol deviation

Protocol deviation refers to any non-compliance with the clinical trial protocol design or procedures. Protocol deviation includes the behaviors that have no effect on the rights and interests, safety and benefits of the participants, or the completeness, accuracy and reliability of the trial data, as well as the evaluation on the safety or key indicators of the protocol; and major protocol deviation (protocol

violation) includes affecting the rights, interests, safety and benefits of participants, or the integrity, accuracy and reliability of test data, as well as the evaluation of endpoints.

Participants with major protocol deviations should be given special attention, and safety information should be collected to ensure their safety. For any protocol deviation occurring during the study, investigators shall record it in detail, report it to the principal investigator and the sponsor, and submit it to the EC as required. The principal investigator and the sponsor shall determine whether participants with protocol deviations will continue to complete the subsequent study procedures.

The list of major protocol deviations includes (but is not limited to):
(1) Failure to obtain informed consent from the participants' guardians;
(2) Enrollment of participants who do not meet the inclusion criteria or meet the exclusion criteria;
(3) Incorrect vaccination of participants with the trial vaccine;
(4) Report serious adverse events (SAEs) without the specified time;
(5) Vaccination with any influenza vaccine other than the trial vaccine during the study period;
(6) Vaccination of the trial vaccine outside the window period;
(7) Blood collection outside the window period;
(8) The interval between vaccinations does not meet the requirements (including emergency vaccination in response to urgent events).

8. Participants

8.1 Inclusion criteria

(1) Children in stable health aged 6-35 months;
(2) Children’s guardians are able to provide legal identity documents and immunization certificates;
(3) Obtain the written informed consent of the participant's guardian;

8.2 Exclusion criteria for the first dose of vaccination

(1) History of the seasonal influenza vaccine for the 2023-2024 influenza season, or have a vaccination schedule during the trial;
(2) History of seasonal influenza in the past 6 months;
(3) Axillary temperature >37.0°C before vaccination;
(4) History of allergy to the vaccine or vaccine ingredients, and have serious adverse reactions to the vaccine, such as urticaria, dyspnea, and angioneurotic edema;
(5) Congenital malformations or developmental defects, genetic defects, severe malnutrition, or asthma;
(6) Serious chronic diseases (Down's syndrome, diabetes, or sickle cell anemia);
(7) Severe nervous system disorder (epilepsy, seizure or convulsion) or psychotic disorder;
(8) Autoimmune diseases (e.g., systemic lupus erythematosus) or immunodeficiency/immunosuppression (e.g., AIDS, post organ transplant);
(9) History of thyroidectomy, asplenia, functional asplenia, and asplenia or splenectomy under any circumstance;
(10) Coagulation abnormalities (such as coagulation factor deficiency, coagulation disease and platelet disorder), obvious bruise or coagulation defects from a doctor's diagnosis;
(11) Has received continuous treatment with corticosteroids or other immunosuppressants (excluding corticosteroid spray therapy for allergic rhinitis and surface corticosteroid therapy for

acute uncomplicated dermatitis) and cytotoxic therapy for ≥14 days in the past 6 months;
(12) Acute diseases in recent 3 days;
(13) Has received blood products in past 3 months;
(14) Has received other investigational drugs within 30 days;
(15) Has received live attenuated vaccines within 14 days or subunit or inactivated vaccine within 7 days;
(16) Has participated in other clinical trials before enrollment and is within the follow-up period, or plan to participate in other clinical trials during the clinical trials;
(17) Any other factors that are inappropriate for participation in the clinical trial by investigator's judgment.

8.3 Exclusion criteria for the second dose of vaccination

If one of the following (1) to (4) conditions occur, the participant is not allowed to continue vaccination at the discretion of the investigator. If one of the following (5) to (6) conditions occur, the investigator shall determine whether the participant continues vaccination. If one of the following (7) to (10) conditions occur, the participant may receive postponed vaccination within the time window specified in the protocol.

(1) Influenza vaccine other than the investigational vaccine is used during the study;
(2) Any SAEs related to vaccination of participants occur;
(3) Severe allergic reaction or hypersensitivity reaction occurs after vaccination (including urticaria/rash within 30 min after vaccination);
(4) Any confirmed or suspected autoimmune or immunodeficiency disease occurs, including human immunodeficiency virus (HIV) infection;
(5) Acute onset of chronic disease or emerging chronic disease occurring after vaccination;
(6) Other reactions occur as judged by the investigator, including severe pain, severe swelling, severe limitation of motion, persistent hyperpyrexia, severe headache or other systemic or local reactions;
(7) An acute disease occurs at the time of vaccination (acute disease is a moderate or severe disease with or without pyrexia);
(8) Axillary temperature＞37.0°C;
(9) The subunit vaccine or inactivated vaccine is inoculated within 7 days, or the live attenuated vaccine is inoculated within 14 days;
(10) Any other factors that are inappropriate for vaccination by investigator's judgment.

8.4 Criteria for withdrawal and termination

(1) The participant's guardian request to withdraw from the clinical trial;
(2) Occurrence of intolerable adverse events, whether it is related to the experimental drug or not;
(3) The health condition of the participant does not permit participation in this study;
(4) Upon any abnormal clinical manifestations, the participants will be determined by the investigator whether they are related to the vaccine and whether the participants will discontinue the clinical trial early;
(5) Any other causes judged by the investigators.

If the participant who discontinues the clinical trial early has received the test vaccine, the clinical trial data of the participant will be used for safety analysis. The participants cannot be replaced

during the study. After withdrawal or discontinuation of a trial in a participant who has received the clinical test vaccine, the investigator shall provide the necessary guidance to the participant in any clinical conditions that may arise in connection with the trial and continue the follow-up until the definitive diagnosis established/stable condition/recovery.

9. Study procedure

9.1 Schedule of activities

Table 10 Schedule of activities

Visit Number	0	1	2	3	4	5	6
Visit date	D-14-0	D0	D7[d]	D28[d]	D35[d]	D56[d]	D208[d]
Preliminary notice, participant enrollment	X						
Informed consent		X					
Demography		X					
Medical history collection		X					
Physical examination		X					
Screening by the inclusion/exclusion criteria[a]		X		X			
Blood collection		X				X	
Vaccination[b]		X		X			
The participants or their guardians/caregiver record safety observations on the diary card/contact card[c]		X	X	X	X	X	
Monitoring of adverse reactions/events (including grade 3 and above adverse events, SAE, AESI)[c]		X	X	X	X	X	X
Record of the concomitant medication/the vaccine usage[c]		X	X	X	X	X	X

a) Screening by inclusion/exclusion criteria should be performed before each dose of vaccination.
b) The participants will be observed for 30 minutes to confirm occurrence of adverse event, especially that of acute anaphylaxis; and they will be followed up as required.
c) Safety observation includes evaluation of the adverse reactions/events and body temperature measurements, the latter of which should be taken daily from day 0 to 7 after the vaccination or when the fever is suspected. The participants record the safety observations on their diary cards within 28 days of each dose of vaccination, and the investigators shall interview the participant regularly to verify and document the adverse events and concomitant medication/the vaccine usage.
d) Please refer to the "Visit plan" for the time window.

Visit plan:

Visit 0 -day -14~0 - Preliminary notice, participant enrollment;

Visit 1 -day 0 - Enrollment of the eligible participants, blood collection and vaccination of the first dose;

Visit 2 -day 7 (+3 days) after the first dose - verify the safety observation, medication and other vaccination records;

Visit 3 -day 28 (+15 days) after the first dose - verify the safety observation, medication, other vaccination records and vaccination of the second dose;

Visit 4 -day 7 (+3 days) after the second dose - verify the safety observation, medication and other vaccination records;

Visit 5 -day 28 (+15 days) after the second dose - verify the safety observation, medication, other vaccination records and blood collection;

Visit 6 -day 180 (+30 days) after the second dose - verification of SAE and AESI observations, records of concomitant medication use, and other special circumstances through telephone follow-up.

During the clinical trial, if an SAE or AESI occurs, the participant's guardian shall be contacted to assist in providing the corresponding medical records, and efforts shall be made to complete the information collection. If it is indeed impossible to collect the information, "unknown" shall be noted in the relevant information section of the original records.

9.2 Enrollment and informed consent

Distribute the recruitment notice to the guardians of potential participants. Explain the informed consent to the participant in detail. On the basis of voluntary participation, the informed consent will be signed by the participant’s guardian and the investigation physician in duplicate; and the participant holds the duplicate. Participants will be recruited in accordance with "8.4 Study Plan".

9.3 Screening and randomization

Volunteers recruited will undergo physical examination with no abnormalities and be screened against inclusion/exclusion criteria. The screening number will be formatted as "S + on-site code + screening sequence number", for example, "SZ0001". Eligible participants after screening will be enrolled and assigned a unique study number in the order of enrollment. Once a study number is assigned, it shall not be reassigned to any other subject. For researchers' numbers of participants, see "7.5.1 Randomization".

9.4 Vaccination

The information of participant and trial vaccine should be checked before the vaccination, and only those who meet the inclusion and exclusion criteria of this clinical trial protocol can be vaccinated. Please refer to “5.5 Route and Schedule of Vaccine Administration” for Immunization Schedule.

9.5 Safety follow-up observation

Observe the participants on-site for adverse events within 30 minutes after each dose of vaccination; and give the participants the diary cards after each dose to record the solicited adverse events (systemic and local) from day 0 to 7 after the vaccination and unsolicited adverse events from day 0 to 28 after the vaccination. Investigators will explain the judgment of adverse events, measurement methods, recording methods, precautions, reporting methods, etc., and distribute diary cards, rulers,

and thermometers. They will also train the participant's guardian on using the thermometer, observing adverse events, filling in the diary card, and make an appointment for the return time. Request the subject's guardian to fill out the diary card in detail from day 0 to 28 after the vaccination to record any clinical symptoms, medications and the use of other vaccines.

Conduct systematic observation 0-7 days after each dose of vaccination. The participant's guardian observes the subject's symptoms and signs every day and fill them in the diary cards for the investigators to collect safety observation data from day 0 to 7. From day 8 to 28, record the adverse events by using the method of voluntary reporting from subject's guardian and regular follow-up by investigators; and at day 28, take back the diary cards. Monitoring of SAEs) and AESIs will continue until 6 months after the second of vaccination. SAEs and AESIs from day 29 after the second of vaccination to 6 months after the second will be collected through a combination of telephone follow-up once every 2 months and active reporting by the subject.

The participants should be informed of recording any adverse events at any time. In case of acute allergic reaction, Grade 3 or above adverse event or SAE, they should report to the investigators at any time and the investigators should conduct investigation, verification, and follow-up after learning until the event is resolved so as to finally complete the detailed investigation and follow-up records, which should include following:

- Description of adverse events
- Start time and end time of adverse events
- Severity classification of adverse events
- Correlation with the vaccination
- Laboratory results
- Treatment

If an acute allergic reaction occurs after vaccination, emergency treatment should be provided in time to relieve the pain of the participants as soon as possible. In case of grade 3 or above adverse event or SAE after vaccination, medical advice should be given in a timely manner; if necessary, the relevant on-site responsible personnel may be contacted to activate the green channel for medical treatment. The medication and medical treatments during each follow-up visit should be recorded in detail. If an AESI occurs (see 9.7.1), the investigators should make a judgment based on clinical symptoms and signs. When necessary, clinical samples (such as blood, urine, etc.) should be collected for laboratory tests, relevant questionnaires should be filled out, and follow-up should be conducted until the AESI is resolved.

9.6 Blood sample collection

All participants will have approximately 2-3 ml of venous blood collected before immunization and 28 days after the second of vaccination, respectively. The serum will be separated for antibody detection. The sample numbering rule is "Study Number + Collection Sequence Number".

All samples collected on-site shall be promptly sent to the laboratory, and handover with laboratory personnel shall be completed. Laboratory personnel shall promptly separate the serum from blood samples for serum antibody detection and aliquot them into 2 tubes (Tube A of the blood sample set should contain no less than 0.5 ml for serum antibody detection; Tube B is the backup serum). After serum separation, it should be promptly frozen and stored at -20°C or below. Records should be kept for sample handover, separation, and storage. For all submitted samples, a specimen

submission record should be filled out, and temperature control records during the submission process should be retained.

9.7 Safety Evaluation

9.7.1 Index for the safety observation

Solicited local adverse events (at the vaccination site): tenderness/pain, induration, swelling, erythema, rash, pruritus.

Solicited systemic adverse events (including vital signs) (non-vaccination site): fever (axillary temperature), acute allergic reaction, skin and mucosal abnormalities, diarrhea, anorexia, vomiting, nausea, cough, fatigue, irritability, lethargy.

See "9.7.7 Safety Evaluation Criteria" for details.

Adverse events of special interest (AESI): include Guillain-Barré Syndrome (GBS), convulsion, encephalitis, myelitis, neuritis, Bell's palsy, vasculitis, thrombocytopenia.

9.7.2 Definition of adverse event/reaction

Vaccine safety will be evaluated based on the severity of the local adverse events, systemic adverse events and vital signs as well as their relevance to the vaccination.

All adverse medical events occurring during the trial (i.e. from the signing of informed consent) will be collected and recorded.

(1) Adverse event (AE): All adverse medical events that occur in a clinical trial participant after receiving an experimental vaccine may be manifested as symptoms and signs of illness or abnormal laboratory tests, but unnecessarily have any causal relationship with an experimental vaccine.

(2) Adverse reaction: Any harmful or undesirable reaction to humans that may be associated with an experimental vaccine during a clinical trial. A causal relationship between an experimental vaccine and an adverse event is at least a reasonable possibility in which the relevance cannot be ruled out.

(3) Serious adverse event (SAE): Refers to any of the following events occurring in clinical trial participants after receiving the trial vaccine:

- Result in death.
- Is life-threatening: An event where the participant is at risk of death at the time of the adverse event's onset; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalization or prolonged of existing hospitalization: (Routine hospital admission such as for antenatal care, labor & delivery is not an SAE)
- Results in a persistent or significant disability or incapacity
- Results in congenital anomalies or birth defects.
- Any other significant medical events (that may not cause death, be life threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the participant and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or

convulsions that do not result in inpatient hospitalization.

Events not considered as serious adverse events:

- Routine emergency room visits (including 24-hour observation stays);
- Hospitalization for routine outpatient examinations;
- Hospitalization for social reasons: Such as hospitalization of the subject due to lack of care;
- Hospitalization for surgery scheduled before signing the informed consent form, but if the condition worsens requiring early surgery, it is considered an SAE;
- Routine physical examination hospitalization;

Death itself is an outcome and not regarded as an SAE. The primary cause of death (the main adverse event leading to death) shall be recorded and reported as an SAE. "Death" shall be reported as a consequence of the corresponding adverse event. If there is no definite cause for death to report, death itself may be reported as an SAE.

(4) Suspected and unexpected serious adverse reaction (SUSAR): is a suspected and unexpected serious adverse reaction, for which, the nature and severity of the clinical manifestations exceed the existing information provided in the brochure of the experimental drug investigators, manual of the on-market drugs or summary of product characteristics, etc.

(5) Solicited/unsolicited adverse events: in this study, the solicitation period is from day 0 to 7 after each dose of vaccination, and the unsolicited period is from day 8 to 28. The solicited symptoms occurring during the solicitated period are referred to as the solicited adverse events and other symptoms during the solicitated period; and all the symptoms during the unsolicited period are referred to as the unsolicited adverse events.

9.7.3 Outcomes of adverse events

Outcomes of adverse events include: (1) recovered, (2) not recovered, (3) recovered with sequelae, (4) recovering, (5) death and (6) lost/unknown.

9.7.4 Correlation of adverse events and vaccines

The investigators should do their best to explain AE and to evaluate its possible causality, that is, the causality between the investigated vaccine and the alternative causes (such as history of underlying disease and the combined treatment), which applies to all AE, both serious and non-serious.

Causality assessments will be determined by the extent to which the events can be reasonably explained in one or more of the following:

A reasonable temporal relationship between the administration of the trial vaccine and the occurrence of adverse events

Reactions of similar nature have previously been observed in this type of vaccine;

Same events have been reported in the literature with regard to the similar types of vaccine;

The occurrence of the event coincided with the vaccination of the administration of the investigational vaccine; and occur again after the re-administration of the vaccine.

The occurrence of the event can be explained by the effects of concomitant medications (vaccines) or the impact of other treatments

AE Causality should be assessed, by the investigator, to ensure whether there is a reasonable possibility that the AE is caused by the administration of the vaccine, based on the following:

General principles for the correlation judgment:

- ✓ **Certainly related:** there is evidence of vaccination with the experimental vaccines; the occurrence of adverse events and the time sequence of the test vaccine administration are reasonable; adverse events are more likely to be explained by the test vaccines than by other causes; positive results after repeated administration of the test vaccine; adverse events are consistent with the prior knowledge of this type or these types of the vaccine.
- ✓ **Probably related:** there is evidence of vaccination with the experimental vaccines; the occurrence of adverse events and the time sequence of the test vaccine administration are reasonable; adverse events are more likely to be explained by the test vaccines than by other causes.
- ✓ **Possibly related:** there is evidence of vaccination with the experimental vaccines; the occurrence of adverse events and the time sequence of the test vaccine administration are reasonable; the occurrence of the adverse events cannot be ruled out as a result of the test vaccine and possibly may be caused by other reasons.
- ✓ **Unlikely related:** there is evidence of vaccination with the experimental vaccines; adverse events are more likely to be caused by other factors; repeated vaccination tests are negative or uncertain.
- ✓ **Not related:** the participants do not use the test vaccine; or the occurrence of adverse events and the time sequence of the test vaccine administration are unreasonable; or there may be other significant factors for the adverse events.

9.7.5 Management of adverse reactions/events

After vaccination, grade 1 or grade 2 adverse reactions, such as redness, swelling, pain or (and) fever and general malaise etc. can disappear automatically, without any special treatment.

The investigators should conduct the investigation and follow-up on the adverse reactions/events occurred in the participants from the beginning of the immunization to day 28 after the second of the immunization, including medical history, physical examination and necessary laboratory examination, treatment and follow-up until the events are resolved.

9.7.6 Report serious adverse events

(1) Reporting Procedures for Investigators

The study institutions shall establish an emergency response plan for SAE handling. Upon learning of an SAE, regardless of whether it is related to the trial vaccine, the investigator must immediately (within 24 hours as required) fill out the Serious Adverse Event Report Form, send the initial report to the sponsor (via the dedicated pharmacovigilance email for clinical trials: [redacted]), and copy the report administrator. In case of emergency, the 24-hour emergency contact can also be reached [redacted]). Subsequent supplementary information/reports shall be submitted as soon as possible until the event is resolved.

When filling out the Serious Adverse Event Report Form, the investigator shall report the entire situation in writing, including but not limited to the name and relevant description of the adverse event, the occurrence time and type of the SAE, duration, severity, causal relationship with

vaccination, outcome, measures taken (symptomatic treatment), and other relevant clinical and laboratory data.

In case of a death event, the investigator shall report to the sponsor and the EC, and provide the autopsy report and final medical report.

Meanwhile, the clinical trial responsible institution/investigator shall promptly forward the sponsor's latest safety information reports on the clinical trial to the EC.

The EC shall receive SAE reports and other safety information reports, keep abreast of the occurrence and handling of SAEs throughout the clinical trial, and conduct follow-up reviews on the handling and reporting of SAEs during the trial.

(2) Reporting Procedures for the Sponsor

The day on which the sponsor first becomes aware of a valid report is designated as Day 0. After receiving the SAE reported by the investigator, the sponsor's pharmacovigilance personnel shall immediately review the completeness and accuracy of the original data. If necessary, they shall send inquiries to the investigator. At the same time, the sponsor shall analyze and evaluate the report, including the severity, relevance to the trial vaccine, and whether it is an unexpected event.

During the drug clinical trial, for adverse reactions determined to be SUSARs, the sponsor's pharmacovigilance personnel shall submit SUSAR reports to the national drug regulatory authorities and health authorities in accordance with the requirements of the Drug Registration Administration Measures [1], Good Clinical Practice [2], and Standards and Procedures for Rapid Reporting of Safety Data During Drug Clinical Trials [28]. If the investigator and the sponsor cannot reach an agreement on the causal relationship between the adverse event and the drug, and either party judges that the relationship with the investigational vaccine cannot be ruled out, the sponsor shall also submit a rapid report.

The sponsor shall submit SUSAR reports to all investigators, study institutions, and ethics committees participating in the clinical trial. Investigators shall promptly read the reports provided by the sponsor and submit the signed reports to the ethics committee.
For SUSAR that are non-fatal or non-life-threatening, the sponsor shall report them as soon as possible after first becoming aware, but no later than 15 calendar days; for unexpected serious adverse reactions that are fatal or life-threatening, the sponsor shall report them as soon as possible after first becoming aware, but no later than 7 calendar days, and submit and complete follow-up information within the subsequent 8 calendar days.

9.7.7 Grading of Adverse events

The solicited local adverse events and unsolicited systemic adverse events: The classification criteria for solicited adverse events are mainly referred to the “Guidelines for criteria classification for adverse events in clinical trials of prophylactic vaccines” [29] (2019) issued by NMPA, as shown in the table below. The solicited adverse events and unsolicited adverse events with the same symptoms are classified according to the following:

Table 11 Grading of local solicited adverse events

	Grade 1	Grade 2	Grade 3	Grade 4
Tenderness (6-23	Resistance or	Crying upon contact	Persistent crying and	Requiring emergency

months old)	withdrawal upon contact or touch	or touch, but consolable	unconsolable	treatment or hospitalization
Pain (24-35 months old)	Not affect or slightly affect limb activity	Affect limb activity	Affect daily life	Loss of basic self-care ability, or hospitalization
Induration* #, Swelling#	＜2.5cm in diameter	≥2.5cm in diameter and <50% of the vaccinated limb (anatomically the limb where the vaccinated site is located, e.g. upper arm or thigh) in area	≥50% of the vaccinated limb or ulcerated or secondary infection or phlebitis or wound drainage in area	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Redness#, rash* #	＜ 2.5cm in diameter	≥2.5cm in diameter and <50% of the vaccinated limb (anatomically the limb where the vaccinated site is located, e.g. upper arm or thigh) in area	≥50% of the vaccinated limb or ulcerated or secondary infection or phlebitis or wound drainage in area	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Pruritus	Pruritus at the vaccination site, but will be relieved without any treatment or within 48 hours after treatment	Pruritus at the vaccination site, and will not be relieved within 48 hours after treatment	Affect daily life	NA

* In addition to directly measuring the diameter of induration and rash for grading, the progress of the measurements should also be recorded.

The largest diameter or area of the induration, swelling, rash and redness should be measured; and the evaluation and grading should be based on the functional grade and actual measurement results, selecting the higher grading index.

Table 12 Grading of systemic solicited adverse events

	Grade 1	Grade 2	Grade 3	Grade 4
Acute allergic reaction*	Local urticaria (blister), treatment not required	Local urticaria (blister), treatment required; or mild angioedema, treatment not required	Generalized urticaria or angioedema, treatment required; or mild bronchospasm	Allergic shock, or Life-threatening bronchospasm, or laryngeal edema
Skin and mucosal abnormalities	Erythema/pruritus/color change	Diffuse Rashes/maculopapules/dryne ss/desquamation	Blistering/exudation/ desquamation/ulcer	Exfoliative dermatitis involved mucosa, or erythema multiforme or suspected Stevens-Johnsons syndrome
Diarrhea	Slight or transient, 3 ～ 4 times/day; abnormal fecal character, or slight diarrhea lasting shorter than 1 week	Mild or transient, 5～7 times/day; abnormal fecal character, or mild diarrhea lasting longer than 1 week	>7 times/day; abnormal fecal character, or bloody diarrhea, orthostatic hypotension or electrolyte imbalance, intravenous infusion >2L required	Hypotension and shock, hospitalization required
Anorexia	Appetite decreased, without food intake reduced	Appetite decreased, with food intake reduced, but without significant	Appetite decreased, with significant weight loss	Intervention required (eg. tube feeding and for

	Grade 1	Grade 2	Grade 3	Grade 4
		weight loss		parenteral nutrition)
Vomiting	1~2 times/24 hours, without affecting the movement	3~5 times/24 hours, or affect the movement	＞6 times/24 hours, or intravenous infusion required	Hypotensive shock, hospitalization require, or other means of nutrition required
Nausea	Transient (＜ 24 hours), or Intermittent and with food intake generally normal	Persistent nausea leads to food intake reduced (24～48 hours)	Persistent nausea leads to food intake very little (> 48 hours), or intravenous infusion required	Life threatening (e.g. hypotensive shock)
Cough	Transient, no treatment required	Persistent cough, treatment effective	Paroxysmal cough, uncontrollable by treatment	Emergency treatment or hospitalization
Fatigue	Normal movement	Normal movement reduced	Severely affecting the movement and unable to work	Emergency treatment or hospitalization required
Irritability	Mild irritability	Irritable	Unsoothable	NA
Lethargy	Mild lethargy	Drowsiness	Hyporesponsiveness	NA
Vital signs- Fever, axillary temperature (℃)	37.5～＜38.0	38.0～＜39.5	≥39.5	≥39.5, for successively 5 days

*refers to hypersensitivity of Type I.

For the adverse events not included in the above grading tables, the severity of adverse events is to be graded according to the following grading criteria:

Mild (Grade 1): short duration (<48h) or slight discomfort, without affecting the movement and no treatment is required;

Moderate (Grade 2): Mild or moderate movement is limited, and medical attention might be required, but with no or only mild treatment;

Severe (Grade 3): Movement is limited significantly, requiring medical attention and treatment, and might require hospitalization; and

Critical (Grade 4): The critical illness can be life threatening, severely limiting the movement and requiring Intensive Care.

Grade 5: Death

9.8 Concomitant medications and vaccines

9.8.1 Concomitant medications

(1) Upon the occurrence of adverse events during the trials, it is allowed to conduct some necessary treatments and medical treatments.

(2) When severe allergic reactions or life-threatening reactions occur, first aid measures should be taken immediately.

(3) The investigators should record the information about any drug combinations, including names, dosage forms, dosages and time of use, etc.

9.8.2 Concomitant vaccines

(1) Other vaccines shall be administered at least 7 days after receiving the investigational vaccine.

(2) During the trial when emergency vaccines are needed, the participants should get vaccinated, such as rabies and tetanus vaccine.

(3) For concomitant vaccines, detailed information should be recorded, including their names, usages and time of the vaccination.

9.8.3 Record of Concomitant Medications and Vaccines

To understand and rule out the impact of concomitant medications and vaccines administered during the trial on the safety and immunogenicity of the trial vaccine, as well as to collect all possible adverse events that may be related to vaccination, investigators shall guide participants' guardians to record details of medical visits, concomitant medications, and vaccines in the diary card as much as possible. In the event of an SAE or AESI, the participant's guardian shall be contacted to assist in providing the corresponding medical records, and efforts shall be made to complete the information collection. If it is indeed impossible to collect the information, "unknown" shall be noted in the relevant section of the original records.

9.9 Immunogenicity evaluation

The immunogenicity endpoints are SCR, SPR, GMT and GMI of influenza HI antibody of each strain.

9.9.1 Definitions

SCR: The percentages of participants with either a pre-vaccination titer <1:10 and a post-vaccination titer ≥1:40, or a pre-vaccination titer ≥ 10 and a ≥ 4-fold increase in post-vaccination titer

Seroprotection: HI antibody titer is ≥1:40.

9.9.2 Laboratory test method

Micro-hemagglutination inhibition test will be used to test the antibody.

9.10 Data management

9.10.1 Source Data

The source Data shall include the informed consent, diary cards and original records, etc., which record the following basic data:

- Name of the trial and participant number;
- Demographic data;
- Inclusion/exclusion criteria;
- Vaccination record;
- The date of the follow-up and the date the participants terminate the trials;
- Adverse events/reactions and their management and outcome;
- Concomitant medical treatment and other vaccinations;

All data should be recorded in source documents, which should be properly stored in a specialized space by the investigator. The original data should be archived at the study site, which is the basis of the participants' participation in the clinical trials and the true integrity of the data.

The investigators should keep the original records carefully, accurately and timely; and all the collected original data should be recorded on the same day. It is required to fill in the record with a black pen. Any errors should not be altered, but be underlined and fill in the correct next to it, and signed with name and the date.

9.10.2 Design of eCRF

The eCRF is designed in accordance with the trial procedures and flowcharts specified in the protocol. After the first draft is formed, it needs to be jointly reviewed by members of the project team including the sponsor, project manager, medical personnel, data personnel, and statistical personnel. It shall conform to the protocol and relevant laws and regulations, and the version control process shall be completely recorded.

9.10.3 eCRF Completing Guidelines

The eCRF completing guidelines are specific instructions for filling in each page of the eCRF and each data point. It is ensured that the clinical trial center obtains the eCRF and its completing guidelines before enrolling participants, and trains the relevant staff of the clinical trial center on the eCRF filling and data submission process. This process shall be archived and recorded.

9.10.4 eCRF Annotations

The annotated eCRF is the marking of the blank eCRF, which records the position of each data item in the eCRF as well as its variable name and code in the database. All data items in the eCRF need to be annotated.

9.10.5 Database Design

The database shall be established in accordance with the dataset name, variable name, variable type, and variable length in the annotated eCRF, and shall comply with the structure and settings of the standard database as much as possible. After the database is established, database testing shall be conducted, and it can be used only after passing the test.

9.10.6 Permission Allocation

The system administrator shall create accounts for different roles and grant different permissions respectively.

9.10.7 eCRF Filling

Investigators shall collect participants’ data in accordance with the requirements of the study protocol, and accurately, timely, completely, and standardly fill the information into the eCRF based on the original data with reference to the filling guidelines. Any modification to eCRF data must comply with standard operating procedures, and modification traces shall be retained.

9.10.8 Issuance and Resolution of Queries

The data management (DM) department shall formulate a detailed data verification plan. After the verification plan is reviewed and approved by the sponsor, medical personnel, statisticians, project

managers, etc., it shall be signed and confirmed by data administrators, data managers, and the sponsor. After data is entered into the eCRF in the EDC system, the system will verify the data in accordance with the Edit Checks established in the data verification plan. Data with doubts will automatically trigger system queries; data that cannot be set to trigger system queries will be sent manual queries through the EDC. Investigators shall confirm and respond to manual and system queries, and modify incorrect data if necessary until the queries are resolved. If the response fails to resolve the query, data administrators and clinical monitors may issue a re-query for the data point. All traces shall be stored in the EDC database.

9.10.9 Data Modification and Review

Investigators may modify data after verifying it. For modified data, the modification reason shall be filled in the system as prompted. Researchers have the authority to review all final data.

9.10.10 Medical Coding

Participants' medical history, as well as AEs and SAEs during the clinical trial, shall be coded using standard dictionaries. The commonly used standard dictionary is the Medical Dictionary for Regulatory Activities (MedDRA). The dataset after coding shall clearly record the dictionary and version used for coding.

9.10.11 Data Review Meeting

Before database locking, the draft data review report and all data lists shall be prepared. The sponsor, investigators, data management personnel, and statistical analysts shall jointly conduct the final review of the database, including the division of statistical analysis populations in accordance with the clinical trial protocol, verification of records of SAE reporting and handling, etc. After the data review meeting, the data management report and population division plan shall be finalized.

9.10.12 Database Lock and Unlock

Database lock is an important milestone in the clinical research process. The lock process and time shall be clearly documented. Database lock means revoking the permission to edit the database, and no unauthorized account shall be able to operate the database.

If modifications are required after the database is locked, an application shall be submitted. The modification can only be implemented after discussion and signature confirmation by the sponsor, investigators, clinical monitors, and data management personnel, and the reasons for unlocking shall be recorded in detail.

9.11 Statistical Analysis

This section was did not translated from Chinese to English. Please see statistical analysis plan (SAP).

10. Monitoring of the clinical trials

10.1 Responsibilities of the sponsor

The sponsor shall implement and maintains the quality assurance and quality control, responsible for quality management to ensure that the trials are carried out according to local regulations, GCP and the protocol.

10.2 Responsibilities of the investigators

The Principal Investigator should provide management and clear labor division for all personnels in the clinical trials. The investigator should keep the personal data of the participants confidential. Documents provided to the sponsor should be identified only by the participant code and participant number. The identification list of the participants will be maintained by the investigators in their file. In accordance with the GCP principle, the original data of each participant is allowed to be monitored, checked and reviewed.

10.3 Personnel training

Before the trial, the personnel should be trained. The training should include: GCP, clinical trial protocol and SOP, etc. If additional CRAs or investigators are added during the trial, they should be trained individually. Retraining may be conducted as deemed necessary by the sponsor or the Principal Investigator. Each training should be recorded.

10.4 Participant compliance assurance

According to the clinical trial protocol, a succinct, clear and coherent volunteer enrollment notification and informed consent shall be developed.

Train the informed consent explainers to communicate with the volunteers using plain language so as to ensure the volunteers to be fully informed.

Screen the participants strictly according to the inclusion/exclusion criteria.

Follow-up personnel should be responsible and professional, improving their communication skills and affinity through training. During the safety follow-up, take measures to ensure effective contact between the participants and the investigators, timely disposal of any adverse reactions found and provide the relevant health consultations to the participants.

For participants who fail to comply with the visit plan and have not received subsequent vaccine doses for non-safety reasons, investigators shall contact the participants' guardians to complete the subsequent vaccine administration and visits in accordance with the vaccination procedures and visit plan specified in the trial protocol.

The trial site is responsible for ensuring that study visits for each participant are within time window. If a participant misses a scheduled visit, every effort shall be made to contact the participant 's guardian and complete the visit within the specified visit window. If the participant fails to complete the visit within the window, the participant will continue to complete the subsequent scheduled visits.

10.5 Investigational vaccine management

10.5.1 Definition and handling of the cold chain failure

If a temperature 8℃ and above is recorded in the refrigerator storing the vaccine as a cold chain failure, the investigators should promptly handle it in accordance with the relevant operating procedures as soon as possible and report to the sponsor in time. It is shown by the accelerated stability study that the quadrivalent influenza vaccine can be placed under 37℃ conditions for 3 days, with trial results meeting the quality standards. Therefore, when the storage temperature of the

test vaccine is ＞8℃ for the first time, but the maximum temperature is ≤10℃ for less than 60 minutes, the vaccine can continue to be used normally. Otherwise, it is required to decide to stop or continue using the vaccine according to the sponsor's written/email response.

10.5.2 Investigational vaccine receipt

When the sponsor sends the investigational vaccine to the study site, the investigator must sign the vaccine receipt form. This receipt form should briefly state the information for the vaccine acceptance (such as complete package and normal indication of the cold chain system).

Do not use the vaccine if the investigators find that the vaccines are is damaged or spoiled, or has lumpy material that cannot be shaken. Return the vaccines to the sponsor. If the cold chain system is destroyed or the vaccines are frozen during the transportation and storage, the vaccines cannot be used. They should be stored separately and marked "X" on the outer package, and assign special personnel in charge of them before returned to the sponsor.

10.5.3 Investigational vaccine management

The investigational vaccines should be stored separately, managed by specially-assigned person in specially-assigned locking cabinet. The storage conditions should meet the storage requirements of the test vaccines and accept the monitoring of the CRAs. The vaccine warehousing account will record the amounts of vaccines received, administered to the participants and the amount remaining or lost. The investigators will count all the test vaccines at the end of the trials. When the study is completed on-site, the remaining vaccines will be counted and returned to the sponsor.

10.6 Clinical trial sample management

The samples used for antibody detection shall be disposed as the medical waste by the institution responsible for serum testing after completing the test. The backup serum shall be temporarily kept by the on-site trial institution until NIFDC issues the immunogenicity test report and the correct verification. The backup serum may be disposed of through mutual consultation between the sponsor and the clinical trial institution after the conclusion of the project. The serum samples from this clinical trial shall be used exclusively for antibody detection in immunogenicity evaluation. Any use of the samples for other research purposes shall obtain the consent of the subject's guardian and the approval of the EC.

10.7 Clinical trial data storage

The clinical trial data shall be kept in accordance with the requirements of GCP; the sponsor, the responsible institution and the on-site trial institution shall keep the clinical trial data for at least 5 years after the trials.

10.8 Completion criteria for the clinical trials

(1) The samples have been sent to the corresponding testing institution and the test reports have been issued;

(2) All the participants have completed the required fellow-up, and the original clinical trial data and documents have been transferred to the archivist for archiving;

(3) The remaining amount of the test vaccines is accurate and handed over to the sponsor; and

(4) The statistical analysis report and clinical summary report meet the requirements of the sponsor.

11.Ethical approval

11.1 Review and approval

This clinical trial protocol should be approved by the EC. The Principal Investigator should submit the clinical trial protocol and all necessary additional documentation to EC. Upon approval by the EC, the investigators should provide the sponsor with a certificate of approval from EC.

11.2 Implementation of on-site supervision

During the whole process of the trial, EC will monitor whether there are any ethical problems that the participants are damaged and whether the participants are treated and compensated for the damage and receive the corresponding measures, and will evaluate the risk degree that the participants are exposed to.

11.2.1 Informed consent

Ensure that the method of participant selection and the relevant information provided to the participants are complete and understandable and ensure that the informed consent is obtained by appropriate means. Throughout the trials, EC will periodically review the progress of the trials and assess the risks and benefits of the participants.

11.2.2 Benefits

After receiving the trial vaccine, participants may produce protective antibodies to prevent infection by the corresponding types of influenza viruses, with the specific outcome depending on the participants' immune response to the vaccine.

11.2.3 Potential risk and risk minimization

If an adverse event is determined to be related to the vaccination (post-vaccination abscess and rash at the vaccination site), the participant will be treated promptly in accordance with the relevant regulations. If a life-threatening incident occurs, the participant will be immediately escorted to the hospital for treatment and reported.

Under strict supervision, the trained and experienced medical staff will perform the vaccination and the venous blood collection in accordance with the prescribed procedures to minimize the injury and pain (including pain and rare local infection at the venipuncture site) caused by the vaccination and blood collection.

In addition, due to individual differences in immunity, some participants may not acquire immune protection after vaccination.

11.2.4 Participant protection

The clinical trials are conducted at the county/city center for disease control and prevention that are eligible for vaccination. The sponsor will evaluate the trial site in strict accordance with GCP requirements prior to the start of the trials. The environmental facilities of the trial site should meet the requirements of the “Guidelines for Quality Management of Vaccine Clinical Trials (Trial)”. Emergency plan for the participant damage and emergency should be made at the trial site. Qualified and experienced doctors and nurses with relevant qualifications will be assigned in the

medical examination rooms and blood collection rooms in order to strictly adhere to the selection/exclusion criteria and successfully finish the blood collection. The emergency rooms are equipped with appropriate first aid facilities, first aid equipment, first aid medicines and the emergency physicians with the appropriate qualifications and abilities. When any adverse events occur on the trial site, the participant will be treated in the emergency room in a timely manner; and in need of emergency hospitalization, after the condition becomes stable treated on-site, the participant shall be transported to the agreed hospital by the ambulance equipped with necessary first-aid facilities and drugs. The trial site and the local comprehensive hospitals at the county level and above will sign the green channel agreement. Agreement shall be notified to hospital that the participants should receive timely medical treatment during their enrollment, including the personnel responsibilities, contact phone and ambulance route etc, to guarantee the emergency treatment and measures in case of occurrence of any adverse events, so as to ensure the effect communication between the participants and the investigators to swift report and treatment of any adverse events. If any participant needs to be hospitalized for emergency treatment upon the occurrence of any serious adverse event, the agreed hospital shall be able to provide green channel medical services, including medical treatment and hospitalization to ensure the participants to get medical treatment in time. The investigators should follow the progress of the events and complete the investigation records until the end of SAE.

11.3 Confidentiality

Ensure that the participant's personal confidentiality is not to be disclosed, including during the trials and the biometric sampling collection as well as including in any reports and publication. The records of the trial samples shall only include the participant code, sample number, sample collection time and the testing index. Access to the relevant electronic or written copies is strictly limited to the Principal Investigator.

12. Revision of clinical trial protocol

After the sponsor and the investigator have signed the clinical trial protocol, if any protocol is to be revised, all of them should be re-signed and dated by the Principal Investigator and the sponsor, followed by the original protocol.

All the revised protocols should be notified to EC and approved by EC before implementation. At the same time when the protocol is being revised, it should be pointed out whether it is necessary to modify the informed consent and electronic CRF, etc.

13. Data disclosure and publication

After the completion of this clinical trial, if their results need to be disclosed and/or published, the positive results will be disclosed and/or published together with the negative results.

14. Reference

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