CLINICAL TRAIL PROTOCOL

Protocol Title: Immunogenicity and Safety of Quadrivalent Influenza Vaccine In 6 to 35 months population: a Randomized, Double-blind, Parallel-group II phase of clinical trials

Collaborators: Henan Center for Disease Control and Prevention

Sponsor: Shanghai Institute of Biological Products

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BACKGROUD INFORMATION

Since 1978, influenza A (H1N1) influenza A (H3N2) and influenza B virus are commonly circling every season. Then, the trivalent influenza vaccine had been developed against the three kinds of influenza virus (H1N1 and H3N2 and B). According to the result of global influenza surveillance, the World Health Organization has been to predict the next season's pandemic strains for more than 30 years. WHO recommended vaccine components containing two subtypes of A influenza virus (H1N1 and H3N2) and a subtype of B influenza virus (B/Victoria lineage or B/Yamagata lineage). However, since the 2001-2002 influenza season, two strains of influenza B have been circulating together. This made it difficult to predict the dominant strain of influenza B in the next influenza season. Several studies demonstrated that in 7 of the past 13 seasons, major cycling subtype of B virus strains was not included in the vaccine, which significantly reduces the immunological effects of the influenza vaccine. Unincluded subtype B influenza virus caused a potential threat to human beings. To expand the broad protection of the seasonal influenza vaccine and improve the protective effect of the vaccine, many influenza vaccine manufacturers had actively carried out the development of the quadrivalent influenza vaccine, which has become an important direction for the upgrading of influenza vaccine.

At the same time, several studies showed that the influenza vaccine induces a poor immune response in young children. Unvaccinated children below 3 years older were induced by vaccine weaker than unvaccinated children above 3 years older younger or vaccinated children below 3 years older, especially for the B subtype influenza virus. For 6 ~ 35 months old children were recommended vaccination dosage of 0.25 ml antigen of influenza inactivated vaccines. This recommendation was based on the observation in the 1970s of increased adverse reactivity in younger children with an adult’s dosage of whole-virus inactivated influenza vaccines, especially the fever. but at present in the seasonal flu vaccine has been without the use of an inactivated virus. The safety and immunogenicity of 0.5ml and 0.25ml dosage antigen of vaccine were evaluated in 6~35 months children in a large number of clinical trials. The results showed that the high dosage of the split vaccine was well tolerated and could induce a strong immune response. Hence, in 2016, the FDA approved the adult dosage of the influenza vaccine for children above 6 months. Besides, many countries such as Canada, Brazil, Mexico, Finland, and Britain approved dosage of 15μg and

7.5 μg antigen for children below 3 years.

Given the situation of children-using the influenza vaccine, we carried out the clinical trial of the quadrivalent influenza vaccine suitable for children aged 6 to 35 months, which is of great significance for children to prevent influenza in China.

STUDY OBJECT

To evaluate the immunogenicity and safety of 2 doses of quadrivalent influenza virus split vaccine (0.25ml/ dose and 0.5ml/ dose) in healthy population aged 6-35 month., so as to provide a data support for phase III clinical trials.

STUDY DESIGN

A randomized, double-blind, parallel control design was set to enroll 1,980 healthy subjects aged 6-35 months. Subjects were randomly inoculated with experimental vaccine 1, experimental vaccine 2, control vaccine 1 or control vaccine 2 in a ratio of 2:2:1:1 for safety and immunogenicity evaluation. The inoculation time was 0 and 28 days.

Immunogenicity observation

- All subjects were required to take bloody samples for 2 times, each time about 3.0ml, for the detection of influenza virus HI antibody:
- First time: before the first dose;
- Second dose: 28 (+10) days after the second dose.
- After 30 days of safety observation and 28 days of immunogenicity test results after 2 doses of immunization, uncovered blind and the immunogenicity results were statistically analyzed.

Safety observation:

- All adverse events were collected at 0-7 days after immunization, all adverse events at 0-28/30 days, and all serious adverse events (SAE) within 6 months (180 days) after 2 doses of vaccine immunization.

Age	Group	Vaccine	Number	Inoculation（day）	Serum collection (day)	Safety observation

6-35 Month	A1	Experimental Vaccine 1	660	0、28（+10）	0，56（+10）	1. Adverse events within 28/30 days after each dose of immunization were collected; 2. Serious adverse events (SAE) were collected within 6 months after the first dose of inoculation and after the whole course of immunization.
	A2	Experimental Vaccine 2	330			
	B1	Control Vaccine 1	330			
	B2	Control Vaccine 2	660			
Total			1980			

STUDY ENDPOINT

Immunogenicity

(1) 28 days after receiving two doses of vaccine in subjects aged 6-35 months, seroconversion rate of HI antibodies against any subtype of influenza virus in each group.

(2) 28 days after receiving two doses of vaccine in subjects aged 6-35 months, seroprotection rate of HI antibodies against any subtype of influenza virus in each group.

(3) 28 days after receiving two doses of vaccine in subjects aged 6-35 months, GMT and GMI of HI antibodies against any subtype of influenza virus in each group.

Safety

(1) The proportion of all adverse reactions/events in each group through 30 days after the second dose.

(2) The proportion of serious adverse events (SAE) within 6 months after inoculation in each group.

SAFETY OBSERVATION

Systemic adverse reactions: Fever, Diarrhea, constipation, difficulty swallowing, anorexia, vomiting, nausea, muscle pain (not the vaccination site), arthritis, joint pain, headache, new convulsions, cough and difficulty breathing, the vaccination site itching (no skin damage), abnormal skin and mucosa, insomnia, irritability, or inhibiting, acute allergic reaction to pain, fatigue, lack of power, not the vaccination site.

Local adverse reactions: pain, induration, swelling, rash, redness, itching

IMMUNOGENICITY OBSERVATION

Seroconversion: 1:10 is the lowest serum dilution. Prevaccination HI titer < 1:10 and postvaccination titer ≥ 1:40 or a prevaccination titer ≥ 1:10 and at least a 4-fold increase in the postvaccination titer

Seroprotection: Postvaccination titer ≥ 1:40 is considered to have antibody protection.

GMT: geometric mean titer.

GMI: GMT increase fold.

EVALUATION CRITERIA OF SAFETY

The safety evaluation of each group will be divided into three stages. From the first dose inoculation to day 7 after inoculation; From the first dose inoculation to day 21 after inoculation; From the second dose inoculation to day 30 after the second dose inoculation. The first stage is the basis for the follow-up group of the same vaccine, and will be carried out under blind. The latter two stages are the basis for evaluation of vaccine safety, and will be carried out under unblind. If each evaluation is not found level 4 local and systemic adverse reactions associated with vaccine and the total incidence rate of level 3 of local and systemic adverse reactions and abnormal laboratory test which associated with the vaccination is lower than 15%, that the vaccine will be acceptable safety.

EVALUATION CRITERIA OF IMMUNOGENICITY

If seroconversion rate is higher than 40%, the antibody protection rate higher than 70%, GMI more than 2.5 times, the vaccine will be acceptable immunogenicity.

STUDY STATUS

Record Verification: January 2020

Study Start: April 2020

Primary Completion: August 2020

Study Completion: March 2021

ELIGIBILITY

Inclusion Criteria

Healthy infants aged 6-35 months.

Volunteer legal guardian or client informed consent, voluntarily participate in and sign informed consent.

The volunteer legal guardian or client has the ability (non-illiterate) to understand the study procedures, to use a thermometer, scale, and fill in a diary card as required, and be able to complete the clinical study in compliance with the clinical trial protocol.

Exclusion Criteria

The underarm body temperature on the day of enrollment was > 37.0℃.

Have been suffering from influenza within the previous 3 months (confirmed by either clinical, serological or microbiological methods).

Any previous influenza vaccination (registered or experimental) within 6 months or any planned influenza vaccination during the study period.

Allergic to any component of the vaccine, a history of allergic reactions to eggs or gentamicin sulfate.

A history of severe allergy to any vaccine or drug.

Preterm birth (delivered before 37 weeks of gestation), low birth weight baby (birth weight < 2300g for girls, <2500g for boys).

Dystocia, asphyxia rescue, nervous system damage history;

Congenital malformations or developmental disorders, genetic defects, severe malnutrition, etc.

Acute illness, severe chronic illness or acute attack of chronic disease on the day of vaccination;

A history of live attenuated vaccination within 14 days prior to vaccination and a history of other vaccinations within 7 days prior to vaccination;

Patients who received immunoenhancement or inhibitor therapy within 3 months (continued oral or intravenous administration for more than 14 days);

Congenital or acquired immune deficiency, HIV infection, lymphoma, leukemia or other autoimmune diseases;

History of asthma, past two years of instability requiring emergency treatment, hospitalization, intubation, oral or intravenous administration of corticosteroids;

Have received blood or blood-related products;

A history of convulsion, epilepsy, encephalopathy, guillain-barre syndrome, a history of mental illness or a family history;

A history of abnormal coagulation function (such as coagulation factor deficiency,

coagulation disease);

Planning to relocate before the end of the study or to leave for an extended period during the scheduled study visit;

Participating in or planning to participate in other clinical trials in the near future;

The investigators determined that any conditions were inappropriate to participate in the clinical trial.

DATA STATISTICS

Statistics analysis of immunogenicity

The prevaccination and postvaccination geometric mean of antibody and its 95% confidence interval (confidence interval, CI) will be described. The postvaccination serum conversion rate and protection rate of antibody and their 95% CI will be described.

Comparisons will be conducted to evaluate differences in response between study groups using a χ^2 test or Fisher's exact test to compare to the difference of serum conversion rate and protection rate. Statistical significance will be considered at a level of $\alpha = 0.05$ and all tests will be 2-sided.

If serum HI antibody conversion rate is more than 40%; protection rate more than 70%; GMI more than 2.5 folds, the immunogenicity will reach the design requirements.

Statistics analysis of safety

After the first dose inoculation and the second dose inoculation, the number of adverse reactions (rate), number of cases and adverse reaction grade were recorded.
The number of adverse events after inoculation will be described.

Comparisons will be conducted to evaluate differences in response between study groups using a χ^2 test or Fisher's exact test to compare to the difference of adverse events rate. Statistical significance will be considered at a level of $\alpha = 0.05$ and all tests will be 2-sided.

Comparison of compliance

The rate of expulsion and the rate of drug combination will be described.

Comparisons will be conducted to evaluate differences in response between study groups using a χ^2 test or Fisher's exact test to compare to the difference of the rate of expulsion and the rate of drug combination. Statistical significance will be considered at a level of $\alpha = 0.05$ and all tests will

be 2-sided.

APPENDIX I. GRADING STANDARDS FOR SEVERITY OF SAFETY INFORMATION

The Event of clinical response and laboratory abnormalities after vaccination is judged by the China State Food and Drug Administration on "Guidelines for the classification of adverse events in vaccine clinical trials ".

Grading of Local Adverse Events

Local Adverse Reactions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potential Life Threatened (Grade 4)**
Pain	Resistance or withdrawal after contact or touch	Crying after touching or touching, but soothing	There is no comfort in continual crying	Require emergency or hospitalization
Induration	Diameter < 2.5 cm	Diameter ⩾2.5cm, and area < 50% of the inoculated limb (anatomically, the limb where the inoculated site is located, such as the upper arm or thigh)	Area ⩾ 50% of inoculated limb or ulceration or secondary infection or phlebitis or wound drainage	Abscesses, exfoliating dermatitis, and necrosis of the dermis or deep tissue
Redness	Diameter < 2.5 cm	Diameter ⩾2.5cm, and area < 50% of the inoculated limb (anatomically, the limb where the inoculated site is located, such as the upper arm or thigh)	Area ⩾ 50% of inoculated limb or ulceration or secondary infection or phlebitis or wound drainage	Abscesses, exfoliating dermatitis, and necrosis of the dermis or deep tissue

Rash Swelling	<15 mm and does not affect activity	15-30 mm or affect activity	>30 mm or limit daily activity	Gangrene
Itching	Itching at the site of inoculation was relieved by itself or within 48 hours after treatment	The site of inoculation was itchy, which did not relieve within 48 hours after treatment	Affect daily life	N/A

Grading of Systemic Adverse Events

Systemic Adverse Reactions	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potential Life Threatened (Grade 4)
Fever	37.5-38.0℃	38.0-39.5℃	≥39.5℃	
Diarrhea	Mild or transient, 3 ~ 4 stools per day or mild diarrhea lasting for less than 1 weeks	Moderate or persistent, 5~7 times per day, or more than 1 week diarrhea	> 7 times of water stool per day, or blood diarrhea, orthostatic hypotension	Hypotension shock requiring hospitalization
Constipation	Need stool softener and diet adjustment	Need a laxative	Stubborn constipation needs to be dredge manually or use enema	Toxic megacolon or ileus
Dysphagia	Slight discomfort in swallowing	Diet restricted	Diet and conversation are very limited; Cannot eat solid food	No liquid food; Need intravenous nutrition
Anorexia	Decreased appetite, but not reduced food intake	Decreased appetite, decreased food intake, but no significant weight loss	Decreased appetite and significantly decreased body weight	Need for intervention(E.g., gastric feeding, parenteral nutrition)
Vomiting	1~2/24 hours with no impact on activities	3~5/24 hours or restricted activities	>6 times within 24 hours or intravenous rehydration is required	Hospitalize or other routes of nutrition are required because of hypotensive shock.

Nausea	Transient (<24 hours) or intermittent and basically normal food intake	Reduced food intake due to persistent nausea (24~48 hours)	Persistent nausea causes almost no food intake (>48 hours) or requires intravenous fluids	A threat to life(E.g. hypotensive shock)
muscle pain (Non-inoculated site)	Do not affect daily activities	Slightly affect daily activities	Severe muscle pain that severely affects daily activities	Emergency or hospitalize
Arthritis	Mild pain with inflammation, erythema or joint swelling; But without hindrance to function	Moderate pain with inflammation, erythema or joint swelling; Hinder function, but do not affect daily activities	Severe pain with inflammation, erythema or joint swelling; Impact on daily activities	Permanent and/or incapacitated joint injury
Arthralgia	Mild pain, no impairment of function	Moderate pain; Painkillers and/or pain are required to interfere with function, but do not affect daily activities	Severe pain; The need for painkillers and/or pain affects daily activities	Impotence pain
Headache	No effect on daily activities, no treatment required	Having a temporary effect on daily activities and may require treatment or intervention.	Seriously affecting daily activities, requiring treatment or intervention	Stubbornness, emergency or hospitalize
New convulsions	Duration of convulsions < 5 minutes, and status after convulsions <24 hours	Duration of convulsions ≥5~< 20 minutes, and status after convulsions <24 hours	Duration of convulsions ≥ 20 minutes or status after convulsions >24 hours	Prolonged and multiple convulsions (e.g., status

				convulsions) or difficult to control (e.g., intractable epilepsy)
Cough	Transient, no treatment required	Keep coughing	Coughing, uncontrollable treatment	Emergency or hospitalize
Difficulty in breathing	Moving difficulty in breathing	Normal activity difficulty in breathing	Rest time difficulty in breathing	Difficulty in breathing, need oxygen therapy, hospitalize or assisted breathing
Pruritus at non-inoculated site (no skin damage)	Slightly itchy, not affecting or slightly affecting everyday life	Itching affects everyday life	Pruritus makes daily life impossible	NA
Abnormal mucous membrane of skin	Erythema/pruritus/color Change	Diffuse rash/maculopapular rash/dry/detritus	Blister/exudate/desquamate/ulcer	Exfoliative dermatitis involving mucosa, or erythema multiforme, or suspected Stevens-Johnsons syndrome
Insomnia *	Mild difficulty in falling asleep, not affecting or slightly affecting daily life	Moderate difficulty in falling asleep, affecting everyday life	Severe sleep difficulty, severe impact on daily life, need to be treated or hospitalize	NA
To provoke or inhibit.	Mild irritability or mild inhibition	Irritable or sleepy.	Unable to soothe or react poorly	NA

Acute allergic reaction **	Local urticaria (blister), no treatment required	Local urticaria, requiring treatment or mild angioedema, no treatment required	Extensive urticaria or angioedema requiring treatment or mild bronchospasm	Anaphylactic shock or life-threatening bronchospasm or laryngeal edema
Fatigue, fatigue	Do not affect daily activities	Affect normal daily activities	Seriously affect daily activities, unable to work	Emergency or hospitalize
Pain at non-inoculated site #(Location identified at the time of reporting)	Mild pain, not affecting or slightly affecting daily life	Pain affects everyday life	Pain does not work in everyday life	Disabling pain, loss of basic self-care

Grading of Blood Biochemical

Serum	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potential Life Threatened (Grade 4)
Elevated liver function caused by AST ALT, influence factors	1.25-2.5 *ULN	2.6-5*ULN	5.1-10*ULN	>10*ULN
Creatinine	1.1-1.5*ULN	1.6-3.0*ULN	3.1-6*ULN	>6*ULN
Urea nitrogen (BUN)	1.25-2.5 *ULN	2.6-5*ULN	5.1-10*ULN	>10*ULN
Bilirubin: elevation caused by factors, but functional examination is normal	1.1-1.5*ULN	1.6-2.0*ULN	2.0-3.0*ULN	>3.0*ULN
Bilirubin: the increase caused by the factors associated with the increase of liver function test index	1.1-1.25*ULN	1.26-1.5*ULN	1.51-1.75*ULN	>1.75*ULN

Grading of Blood Routine

Detection indicators	Level 1	Level 2	Level 3	Level 4
Liver function (ALT, AST increased)	1.25~<2.5 ×ULN	2.5~<5.0×ULN	5.0~<10×ULN	≥10×ULN
Total bilirubin increased (Mg/dL; Mmol/L)	1.1~<1.6×ULN	1.6~<2.6×ULN	2.6~5.0×ULN	≥5.0×ULN

Grading of Urine Routine

Test Indicators/Grades	Level 1	Level 2	Level 3	Level 4
WBC (109/L)	11~<13	13~<15	15~<30	≥30
WBC (109/L)	2.000~2.499	1.500~1.999	1.000~1.499	<1.000
Lymphocyte Decrease (LY, 109/L)	0.75~1.00	0.5~0.749	0.25~0.49	<0.25

Neutropenia (ANC, 109/L)	0.800~1.000	0.600~0.799	0.400~0.599	<0.400
Eosinophils (Eos, 109/L)	0.65~1.5	1.51~5.0	>5.0	High eosinophil syndrome
Thrombocytopenia (PLT, 109/L)	NA	50~75	25~49	<25
Low hemoglobin (g/dL)	9.5~10.4	8.5~<9.5	6.5~<8.5	<6.5

APPENDIX II. THE RELATIONSHIP BETWEEN ADVERSE EVENTS AND TRIAL VACCINES

Absolutely unrelated: Because of other factors lead to adverse events, there is evidence that adverse events are caused by other causes, not related to vaccination.

Possible unrelated: Adverse events probable be caused by other factors, such as the clinical status of the subjects, other treatment or concomitant medication, inconsistent with the known adverse reactions of vaccination.

Quite possible related: Adverse events are consistent with known information of vaccine, and there is a causal relationship with the vaccine, not by other factors, such as the clinical status of the subjects, or other treatment with medication.

Possible related: Adverse events are consistent with known information of vaccine, and there is a causal relationship with the vaccine, not by other factors, such as the clinical status of the subjects, or other treatment with medication.

Related: Adverse events are consistent with known information of vaccine, and there is a causal relationship with the vaccine, not by other factors, such as the clinical status of the subjects, or other treatment with medication. In addition, adverse events will be repeated when subjects are tested with the vaccine.