CLINICAL TRAIL PROTOCOL

Protocol Title: Immunogenicity and Safety of a Quadrivalent Influenza Vaccine: a Randomized, Blind, a Phase III Clinical Trial.

Collaborators: Jiangsu Center for Disease Control and Prevention

Sponsor: Shanghai Institute of Biological Products

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BACKGROUD INFORMATION

Influenza is an acute, highly infectious and rapid respiratory infectious disease caused by the influenza virus, which is one of the important causes of human death. Influenza viruses are classified into three subtypes A, B and C. Influenza A and influenza B viruses, particularly influenza A viruses, are prevalent in the population and in the form of seasonal influenza, while influenza C viruses are uncommon and cause only mild symptoms. Influenza A viruses can be divided into different subtypes according to the two surface antigens hemagglutinin (HA) and neuraminidase (NA) of the virus.

Currently, influenza A viruses causing seasonal influenza are mainly influenza viruses of the H1N1 subtype and H3N2 subtype. The investigation showed that the B/Victoria lineage dominated the B/Yamagata strain from 1940 to 1980, while the B/Yamagata lineage dominated from 1987 to 1989. But since 2001, two different strains of B influenza virus B/Victoria/2/87-like and B/Yamagata/16/88-like have been circulating at the same time.

In February 2012, the world health organization (who) for the first time suggested that t the seasonal flu vaccine should contain two kinds of B lineage strain proposal. Since 2013-2014, flu season in the northern hemisphere, WHO began to recommend quadrivalent vaccine, to add another subtype B influenza virus in trivalent vaccine, in order to achieve better protection effect. In recent years, the development of quadrivalent influenza vaccine has become an important trend of influenza vaccine.

STUDY OBJECT

First objects:

To evaluate the immunogenicity of quadrivalent influenza vaccine 30 days in healthy population years aged 3 years and above, according to investigate antibodies at day 30 after one inoculation

Secondary objects:

To evaluate the safety of quadrivalent influenza vaccine 30 days in healthy population years aged 3 years and above, according to investigate occurrence of adverse events for 30 days after one inoculation.

To prove conversion rate and geometric mean titer (GMT) of quadrivalent influenza vaccine being non-inferior for shared strains to trivalent influenza vaccines.

STUDY DESIGN

This will be a randomized, blind, one-center, active comparator study conducted by Jiangsu Center for Disease Control and Prevention.

There will be three groups. Group A (quadrivalent influenza vaccine), Group B (trivalent influenza vaccine containing B/Victoria lineage) and Group C (trivalent influenza vaccine containing B/Yamagata lineage). Each group will be divided four age groups (3-8 years, 9-17 years, 18-59 years, 60 years and older).

All subjects will be inoculated with the corresponding vaccine at day 0. Then subjects will receive immediate response observation for 30 minutes and systematic safety observation for 7 days after each dose of vaccination. After 7 days of vaccination, the adverse events will be observed by weekly regular follow-up and the subjects' reporting.

All subjects will be collected serum samples at before vaccination and day 30 after vaccination. All serum samples will be detected HI antibody.

STUDY ENDPOINT

Primary endpoint

To detect the serum HI antibody at day 21 after last inoculation as secondary endpoint.

Secondary endpoint

To investigate adverse events (AE) and serious adverse events (SAE) within 30 days after the inoculation as primary endpoint.

SAFETY OBSERVATION

Systemic adverse reactions: fever, headache, fatigue, fatigue, nausea, vomiting, diarrhea, muscle pain, cough, allergy (anaphylactic shock, urticaria, vascular edema, etc.).

Local adverse reactions: pain, redness, swelling, induration, rash (injection site), itching, skin mucous membrane.

The events (hospitalization, hospitalization time, disability of work ability, life or death, congenital malformation and so on) occur during clinical trial.

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: October 2018
Study Start: November 2018
Primary Completion: March 2019
Study Completion: June 2019

ELIGIBILITY

Inclusion Criteria:

Over the age of three years,healthy population
Subjects/ (and the guardian) informed consent, voluntarily participated and signed the

informed consent form, with the ability to use thermometers, scales and to fill in diary cards as required

To comply with the requirements of clinical trial program

Temperature≤37.0℃ on day of enrollment

Exclusion Criteria:

A history of influenza virus infection or suspected infection Abnormal blood routine, blood biochemistry and urine routine examination indexes in last three months

Any prior administration of influenza vaccine in last six months

Allergy to any component in the vaccine, especially for egg allergy

Allergy history of any previous vaccination or drug

Acute episodes of chronic illness or acute illness on the day of vaccination

Received a live vaccine within fourteen days prior to receiving the vaccine, or received a subunit or inactivated vaccine within seven days

Congenital or acquired immune deficiencies, or treatment with immunosuppressive agents, such as long-term treatment with systemic corticosteroids

Suffering from severe chronic diseases (such as Down's syndrome, diabetes, sicklemia or neurological disorders, Green's Barre syndrome)

Asthma, required urgent treatment in last two years

The blood products were received prior to the acceptance of the vaccine

Known or suspected of respiratory disease, acute infection or chronic disease active period, HIV infection, cardiovascular disease, severe hypertension, malignant tumor during treatment, skin diseases

History of epilepsy, convulsions, or a family history of psychosis

Abnormal coagulation function (such as coagulation factor deficiency, coagulation disorders, platelet abnormalities), or obvious bruising or coagulopathy

Plan to move or leave the area for an extended period of time before the end of the study

Under anti-tb treatment

Any prior administration of other research medicine/vaccine in last one month

Women are pregnant or in the near future planned pregnancy or pregnancy test positive

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	Do not affect activity	Influence activities or multiple use of non-narcotic analgesics	Affect daily activities or multiple use of narcotic analgesics	Emergency or hospitalization
Induration	<15 mm	15-30 mm	>30 mm	Gangrene or exfoliative dermatitis
Redness	<15 mm	15-30 mm	>30 mm	Gangrene or exfoliative dermatitis
Swelling	<15 mm and does not affect activity	15-30 mm or affect activity	>30 mm or limit daily activity	Gangrene
Rash (injection site)	<15 mm	15-30 mm	>30 mm	
Itching	Injection site itching	Injection of moderate itching	Overall Itching	

Grading of Systemic Adverse Events

Systemic Adverse Reactions	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potential Life Threatened (Grade 4)
Fever	37.1-37.5℃	37.6-39.0℃	>39.0℃	
Headache	No activity, no treatment	Transient, slightly affected activity requiring treatment (multiple use of non-narcotic analgesics)	Severe effects on daily activities, initial anesthetic response	Refractory, repeated anesthetic treatment. Emergency or hospitalization
Fatigue and fatigue	The normal activity is less than 48 hours, and it did not affect the activity	Normal activity decreased from 20% to 50% > 48 hours, slightly affecting activities	Normal activity decreased by more than 50%, seriously affecting daily activities, unable to work	Unable to take care of oneself, emergency or hospitalization
Nausea and vomiting	1~2 times per 24 hours, intake is normal and does not affect the activity	2 ~ 5 times per24 hours, intake is significantly reduced, or limited activity	> 6 times within 24 hours, no obvious intake, the need for intravenous infusion	requires hospitalization or other nutrition because of hypotension, shock
Diarrhea	Mild or transient, 2 ~ 3 stools per day or mild diarrhea lasting for less than 1 weeks	Moderate or persistent, 4~5 times per day, or more than 1 week diarrhea	> 6 times of water stool per day, or blood diarrhea, orthostatic hypotension	Hypotension shock requiring hospitalization
Myalgia	Not affecting daily activities	Muscle tenderness at the non injection site slightly affects daily activity	Severe muscle tenderness seriously affects daily activities	The symptoms are obvious, muscle necrosis, emergency or hospitalization

Cough	Transient, without treatment	Persistent cough, effective treatment	Paroxysmal cough, treatment can not control	Emergency or hospitalization
Allergy	Pruritus without skin rash	Local urticaria	Extensive urticaria, vascular edema	Severe allergy
Other adverse or clinical adverse reactions (based on the correspondi ng criteria)	Do not affect activities	Slightly affects activities without drug treatment	Serious impact on daily activities requires drug treatment	

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.