CLINICAL TRAIL PROTOCOL

Protocol Title: Immunogenicity and Safety of an Alum-adjuvanted Inactivated H7N9 Influenza Vaccine: a Randomized, Blind, Placebo-controlled, a Phase I/II Clinical Trial.

Collaborators: Henan Center for Disease Control and Prevention

Sponsor: Shanghai Institute of Biological Products

Document Date: October 17, 2017

BACKGROUD INFORMATION

The first human infection with novel avian-origin influenza A (H7N9) was reported in China in March 2013. As of 26th October 2017, a total of 1564 laboratory-confirmed human H7N9 infectious, including at least 612 deaths, with high mortality (approximately 40%), have been reported to World Health Organization (WHO) since early 2013. Recently, the activity of the H7N9 virus appears increasing trend.

Despite the fact that the H7N9 virus has not yet been able to sustain human to human transmission in the current, it is difficult to judge its epidemic trend. A lot of researches demonstrates that the novel H7N9 the viruses have been evolving from low pathogenic into highly pathogenic and process a potential pandemic threat to public health worldwide due to lack of pre-existing immunity in humans. Therefore, it is prudent to develop strategies to prepare for H7N9 pandemic outbreak. Vaccination is currently considered as effective strategy of controlling influenza viruses with pandemic potential.

In 2013 May, three vaccine strains, A/Shanghai/2/2013 (IDCDC-RG32A, NIBRG-267) and A/Anhui/1/2013 (NIBRG-268), were used for vaccine development. Shanghai Institute of Biological Products (SIBP) carried out research and development of H7N9 avian influenza virus vaccine. The whole and split virus inactivated vaccines were taken as the research direction of the traditional influenza vaccine strategy. However, previous studies indicated the H7-containing vaccines were poor immunogenic in humans and difficult to induce effectively protective antibodies. Adjuvantation is universally regarded as effective means of immune-enhancing and antigen-sparing. As aluminum is the only approved adjuvant in China, we added the aluminum hydroxide adjuvant in the vaccine, to enhance the immune response. At present, vaccine produced by SIBP has received clinical approval, ready to carry out phase I/II clinical trials, safety and immunogenicity of the vaccine will be evaluated by clinical trials.

STUDY OBJECT

First objects: to evaluate the safety of different doses (7.5 μg, 15 μg and 30 μg HA antigen) of influenza A (H7N9) virus inactivated vaccine in healthy population aged 12 years and over, according to investigate occurrence of adverse events for 30 days after two inoculations.

Secondary objects: to evaluate the immunogenicity of different doses (7.5 μg, 15 μg and 30 μg HA antigen) of influenza A (H7N9) virus inactivated vaccine in healthy population

aged 12 years and over, according to investigate antibodies at day 21 after two inoculations.

STUDY DESIGN

This will be a randomized, blind, one-center, placebo control study conducted by Henan Center for Disease Control and Prevention. There will be two phases. The vaccine dosage and the immunization procedures of phase Ⅱ will be determined according to the safety and immunogenicity results of phase Ⅰ.

There will be three dosage groups of H7N9 influenza virus inactivated whole virus vaccine: Group A (7.5 μg HA antigen H7N9 influenza vaccine); Group B (15 μg HA antigen H7N9 influenza vaccine) and Group C (30 μg HA antigen H7N9 influenza vaccine). Every group is divided into subgroups according to different age. Subgroup 1 (group of adults aged 18 years and above); Subgroup 2 (group of youth aged 12~17 years). Each subgroup will be treated with 2 placebo controls (Aluminum Hydroxide and PBS).

All subjects will be inoculated with the corresponding vaccine at 0 and 21 days. 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 the first dose of vaccination, day 21 after the first dose of vaccination and day 21 after the second dose of vaccination. All serum samples will be detected HI antibody. See Table below.

Group	Age	Dosage and Number of subjects		Inoculation	Serum collection	Safety observation
A1	≥18 years	7.5 μg per dose	30	Day 0,21 (+7 days)	Day 0,21 (+7),42(+10)	The local and systemic AE and SAE will be observed for 6 months after inoculation.
		Placebo (Alum)	15			
		Placebo (PBS)	15			
A2	12~17 years	7.5 μg per dose	30			
		Placebo (Alum)	15			
		Placebo (PBS)	15			
B1	≥18 years	15 μg per dose	30			
		Placebo (Alum)	15			
		Placebo (PBS)	15			
B2	12~17 years	15 μg per dose	30			
		Placebo (Alum)	15			
		Placebo (PBS)	15			
C1	≥18 years	30 μg per dose	30			
		Placebo (Alum)	15			

		Placebo (PBS)	15			
C2	12~17 years	30 μg per dose	30			
		Placebo (Alum)	15			
		Placebo (PBS)	15			
Total	6 subgroups	/	360	/	/	/

STUDY ENDPOINT

Primary endpoint

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

Secondary endpoint

To detect the serum HI antibody at day 21 after first and second inoculation as secondary 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.

GROUP PROCESS

In order to protect the rights of the subjects, each group will be carried out after evaluating the safety of last group. The sequence of inoculation is from low dosage to high, from older age to young. The flow sequence is shown in table below.

Day	Group A	Group B	Group C
0	Group A1 First Dose		
8	Group A2 First Dose	Group B1 First Dose	
16		Group B2 First Dose	Group C1 First Dose
21	Group A1 Second Dose		
24			Group C2 First Dose
29	Group A2 Second Dose	Group B1 Second Dose	
37		Group B2 Second Dose	Group C1 Second Dose
45			Group C2 Second Dose
51	Complete 30 days observation of Group A1		
59	Complete 30 days observation of Group A2	Complete 30 days observation of Group B1	
67		Complete 30 days observation of Group B2	Complete 30 days observation of Group C1
75			Complete 30 days observation of Group C2
201	Complete 180 days		

	observation of Group A1 (collect SAE)		
209	Complete 180 days observation of Group A2 (collect SAE)	Complete 180 days observation of Group B1 (collect SAE)	
217		Complete 180 days observation of Group B2 (collect SAE)	Complete 180 days observation of Group C1 (collect SAE)
225			Complete 180 days observation of Group C2 (collect SAE)

STUDY STATUS

Record Verification: October 2017

Study Start: November 2017

Primary Completion: October 2018

Study Completion: December 2019

ELIGIBILITY

Inclusion Criteria:

Over the age of 12 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, receive blood test before and after immunization and cooperate with follow-up

Exclusion Criteria:

A history of influenza A (H7N9) virus infection or suspected infection

Abnormal blood routine, blood biochemistry and urine routine examination indexes

Allergy to any component in the vaccine (allergy history of any previous vaccination), especially for egg allergy

History of asthma, history of thyroid resection, vascular nerve edema, diabetes mellitus, and hypertension cannot be controlled by medicine, liver and kidney diseases or malignant tumor history

Suffered from any serious illness, such as cancer, autoimmune disease, progressive atherosclerotic disease or complications of diabetes, chronic obstructive pulmonary disease, need oxygen therapy for acute or progressive liver or kidney disease, congestive heart-failure etc.

History of signs disease or symptoms of neurological symptoms

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

Acute attacks of various acute or chronic diseases in the past 7 days

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

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

No spleen, functional absence of spleen, and splenectomy or splenectomy without any condition

Autoimmune diseases or immunodeficiency have been treated with immunosuppressive agents in the past 6 months

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

The blood products were received within 3 months prior to the acceptance of the vaccine

Received a live vaccine within 14 days prior to receiving the vaccine, or received a subunit or inactivated vaccine within 7 days

Fever within 3 days prior to vaccination, axillary temperature ≥38℃

Fever When inoculating vaccine, axillary temperature >37.0℃

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

Participants in another clinical trial

The researchers believe that there may be any impact on the assessment of the 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	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	
Mucocutaneous	Redness	Diffuse, papular rash, dry, desquamation	Blister, damp, desquamation or ulcer	Peeling dermatitis, involving mucous membranes, or erythema multiforme, or suspected

				Stevens-Johnso ns syndrome

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	

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	1.1-1.5*ULN	1.6-2.0*ULN	2.0-3.0*ULN	>3.0*ULN

normal				
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

Blood	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potential Life Threatened (Grade 4)**
Leukocyte high(cells/mm^3)	>13000	13,000-15,000	15,000-30,000	>30,000
Leukocyte low (cells/mm^3)	2500-3500	1500-2499	1000-1499	<1000

Grading of Urine Routine

Urine	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potential Life Threatened (Grade 4)**
Protein	trace	1+	2+	>2+
Urine sugar	trace	1+	2+	>2+

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.