Protocol

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This trial protocol has been provided by the authors to give readers additional information about the work.

Efficacy and safety of the RBD-dimer-based Covid-19 vaccine ZF2001 in adults

This supplement contains the following items:

1. Original protocol (V1.0), final protocol (V1.3), summary of amendments

2. Statistical analysis plan (V1.0) (no amendments for this document)

Study title: A Phase III Randomized, Double-blind, Placebo-controlled Clinical Trial in 18 Years of Age and Above to Determine the Safety and Efficacy of ZF2001, a Recombinant Novel Coronavirus Vaccine (CHO cell) for Prevention of COVID-19

Protocol number: LKM-2020-NCV-GJ01
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Statement of Confidentiality

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1. Synopsis

Study Title	A Phase III Randomized, Double-blind, Placebo-controlled Clinical Trial in 18 Years of Age and Above to Determine the Safety and Efficacy of ZF2001, a Recombinant Novel Coronavirus Vaccine (CHO Cell) for Prevention of COVID-19
Generic title	Phase III clinical trial of Recombinant Novel Coronavirus Vaccine (CHO cell)
Investigational product	**Investigational Vaccine:** Name: Recombinant Novel Coronavirus Vaccine (CHO cell) Main ingredients: NCP-RBD from SARS-COV-2 Spike protein, aluminum hydroxide adjuvant. Dosage Form: injection Strength: 0.5mL/vial. It contains 25 μg NCP-RBD proteinBatch number: see the drug inspection report. **Placebo comparator:** Name: Placebo for Recombinant Novel Coronavirus Vaccine (CHO cell) Main ingredients: Aluminum hydroxide adjuvant Dosage Form: injection Strength: 0.5mL/vial. It contains 0.25mg Aluminum hydroxide adjuvant. Batch number: see the drug inspection report.
Indications	For prevention of Coronavirus Disease 2019 (COVID-19) caused by SARS-COV-2 infection
Study Population	Population aged 18 years and above
Research Institutio	

n	
Location of Study	Six sites in Asia, Africa and Latin America
Study Objectives	**Primary objective:** To evaluate the efficacy and safety of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against symptomatic COVID-19 in a population aged 18 years and above. **Secondary objectives:** 1. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against severe and critical COVID-19 in a population aged 18 years and above. 2. To evaluate the immunogenicity and immune persistence of the Recombinant Novel Coronavirus Vaccine (CHO Cell) in a population aged 18 years and above. 3. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) as emergency vaccination against symptomatic COVID-19 in a population aged 18 years and above. 4. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against symptomatic COVID-19 in populations of different age group (18-59 years vs. 60 years and above). **Exploratory objectives:** To explore the immunological surrogate variables of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against COVID-19 in a population aged 18 years and above.
Study Design	Overall design: A randomized, double-blind, placebo-controlled international multicenter clinical trial design will be adopted. A total of 29,000 subjects aged 18 years and above are planned to be recruited, including 750 subjects aged 18-59 years and 250 subjects aged 60 years and above in China; 21,000 subjects aged 18-59 years

and 7,000 subjects aged 60 years and above will be recruited outside China. Safety and immunogenicity will be evaluated among the Chinese subjects, and efficacy, immunogenicity and safety will be evaluated among the subjects outside China. Among them, 750 subjects aged 18-59 and 250 subjects aged 60 and above from outside China and all subjects from China will be selected as the immunogenicity subgroup for immunogenicity bridging study. The efficacy study cohort will set a immunogenicity subgroup of 1000 subjects, of which 500 are given either the investigational vaccine or the placebo (subjects in immunogenicity subgroup participated in the efficacy evaluation at the same time). At the same time, a domestic immunogenicity study cohort will be set up, with a total of 1,000 subjects, of which 500 are given either the investigational vaccine or the placebo. The IgG levels of SARS-COV-2 neutralizing antibody and RBD protein binding antibody will be detected by blood sampling before vaccination, 14 days and 6 months after full course of vaccination to evaluate the immunogenicity and immune persistence.

<table>
<tr><th>Region</th><th>Age group</th><th>Immunogenicity evaluation</th><th>Safety evaluation</th><th>Efficacy evaluation</th><th>Immunization schedule</th></tr>
<tr><td rowspan="2">China</td><td>18 -59 yrs. (750 cases)</td><td rowspan="2">All subjects</td><td rowspan="2">All subjects</td><td rowspan="2">None</td><td rowspan="2">Months 0, 1, 2</td></tr>
<tr><td>60 years and above (250 cases)</td></tr>
<tr><td rowspan="2">Outside China</td><td>18 -59 yrs. (21,000 cases)</td><td>750 cases</td><td rowspan="2">All subjects</td><td rowspan="2">All subjects</td><td rowspan="2">Months 0, 1, 2</td></tr>
<tr><td>60 years and above (7,000 cases)</td><td>250 cases</td></tr>
</table>

	Study population: A total of 29,000 subjects aged 18 years and above, including 28,000 subjects outside China and 1,000 subjects in China. **Study Plan and Implementation:** After signing the informed consent form, the volunteers aged 18 years and above will receive the relevant examinations after an inquiry of the medical history (including COVID-19 history), allergy history and concomitant medications, and demographic data collection by the investigators, including physical examination (skin and mucous membranes, lymph nodes, head, neck, chest, abdomen, spine/limbs), novel Coronavirus (SARS-COV-2) nucleic acid test and antibody test, urine pregnancy (women of childbearing age) test, and vital signs (blood pressure, axillary/oral temperature, pulse) evaluation. Screening eligible subjects will be 1:1 randomly assigned to the experimental group and the placebo control group, and vaccinated as per the 0, 1, 2 month immunization schedule. **Safety evaluation:** **AEs and SAEs:** All adverse events (AEs) up to 30 minutes after each dose of vaccination, all AEs from 0 to 7 days (including both solicited and unsolicited), and all AES from 8 to 30 days (unsolicited) will be collected; All serious adverse events (SAEs) will be collected from the first dose of vaccination to 12 months after the whole vaccination. **Solicited AEs (the following events occurred within 7 days after vaccination):** Injection site (local) adverse events: pain, swelling, induration, redness, rash, pruritus Vital Signs: fever Non injection site (systemic) adverse events: headache, fatigue /

	asthenia, nausea, vomiting, diarrhea, muscle pain (non-injection site), cough, acute allergic reaction, mental disorder (specific symptoms) **ADE/VED (Antibody Dependent Enhancement / Vaccine Enhanced Diseases) risk monitoring:** After vaccination (at least one dose of Investigational product), the subjects shall visit the hospital for hospitalization or isolation according to the local epidemic prevention and control requirements in case of confirmed COVID-19. Special investigation is needed for severe, critical or fatal cases, the DSMB shall analyze based on the results of special investigation whether ADE/VED phenomenon exists. **Efficacy evaluation:** The incidence rate and efficacy of symptomatic COVID-19 14 days after whole vaccination. **Immunogenicity and immune persistence evaluation:** Blood samples (5ml) will be collected before the first dose of vaccination and 14 days and 6 months after the whole course of vaccination to detect neutralizing antibody of SARS-COV-2 and protein binding antibody of receptor binding region (RBD).
Inclusion criteria	1) Population aged 18 years and above; 2) Subjects voluntarily participate in the study and sign the informed consent form; and are able to provide valid identification, and understand and comply with the requirements of the trial protocol; 3) Female subjects of childbearing age agree to use effective contraceptive measures from the beginning of the study to 2 months after full course of vaccination.
Exclusion criteria	(1) Suspected or confirmed as fever within 72 hours before the enrollment, or axillary temperature ≥ 37.3 ° C / oral temperature ≥37.5°C at the day of screening; (2) Diastolic blood pressure ≥ 100mmhg and / or systolic blood

	pressure ⩾ 150mmhg; (3) Patients with previous history of a COVID-19; (4) Detection of SARS-COV-2 nucleic acid or antibody is positive; (5) Those who are suffering from the following diseases: a) With thrombocytopenia, any coagulation dysfunction or receiving anti-coagulatory treatment b) Congenital or acquired immune deficiency or autoimmune disease history; no spleen, or history of splenic surgery and trauma, or receiving immunomodulator treatment within 6 months, e.g., immunosuppressive dose of glucocorticoids (reference dose: equivalent to 20mg/ day of prednisone, over 1 week); Or monoclonal antibodies; Or thymosin; Or interferon etc.; However, topical application (such as ointment, eye drops, inhalers or nasal sprays) is permitted; c) Symptoms related to acute respiratory tract infection (such as sneezing, nasal congestion, runny nose, cough, sore throat, loss of taste, chills, shortness of breath, etc.); d) Cancer patients (except basal cell carcinoma) (6) With a history of serious allergy to any vaccine or any composition of Investigational product (including: aluminum preparations), such as allergic shock, allergic throat edema, allergic purpura, thrombocytopenic purpura, localized allergic necrosis reaction (Arthus reaction), dyspnea and angioneuroedema; (7) Inoculated with subunit vaccine and inactivated vaccine within 14 days before the first dosing of investigational? vaccine, or inoculated with attenuated live vaccine within 30 days; (8) Previous receiving blood transfusion or blood relevant products (including immunoglobulin) within 3 months, or planning to receive such products from the starting of study to <6 months after the whole-course inoculation; (9) Have participated in or are participating in other covid-19 related clinical trials;

	(10) Women in breastfeeding period or in pregnant period (including women at childbearing age with positive result of urine pregnancy test); (11) Considered by investigators as any disease or state possibly making the subject at unacceptable risk; not conforming to the requirements of study protocol; interference of assessment of reactions of vaccine.
Criteria for withdrawal	Withdrawal decided by the investigators: 1) AEs or concomitant conditions that discontinue the trial; 2) Having participated in other clinical trials before the end of this clinical trial; 3) Subjects with other conditions that are not suitable to participate in the clinical study, as considered by the investigator; Withdrawal requested by the subjects: subjects can freely discontinue the study participation at any stage during the study.
Study Endpoints	**Primary endpoints:** (1) The endpoint of efficacy study: The number of symptomatic COVID-19 cases 14 days after whole vaccination. (2) The endpoint of safety study: a. Analysis of adverse events from the first dose of vaccination until 30 days after full course of vaccination: incidence of adverse reactions or adverse events; incidence of grade 3 or above adverse reactions or adverse events; incidence of adverse reactions or adverse events leading to withdrawal. b. Analysis of serious adverse events from the first dose of vaccination until 12 months after full course of vaccination: incidence of serious adverse events; incidence of serious adverse events associated with Investigational product. **Secondary endpoints:** (1) The endpoint of efficacy study:

	a. The number of severe and critical COVID-19 cases 14 days after whole vaccination; b. The number of symptomatic COVID-19 cases 14 days after first dose of vaccination; c. The number of COVID-19 cases of any severity in populations of different age group (18-59 years vs. 60 years and above) 14 days after whole vaccination. (2) Endpoint of immunogenicity and immune persistence study: The level of neutralizing antibody to SARS-COV-2 and IgG level of RBD protein binding antibody at 14 days and 6 months after full course of vaccination. **Exploratory endpoint:** The protective level of neutralizing antibody to SARS-COV-2 and IgG of RBD protein binding antibody against COVID-19 caused by SARS-COV-2 infection.
Research Hypothesis	**Primary study hypothesis:** At least 14 days after full course of vaccination, the vaccine provide better protection against symptomatic COVID-19 than the placebo (The lower bound of the 95% confidence interval is >30%).
Sample Size Considerations	**Sample size calculation based on efficacy study** A large-scale, confirmatory clinical study is needed to evaluate the protective effect of the vaccine on COVID-19 in people aged 18 years and above. The power is calculated based on the assumption of the incidence rate of symptomatic COVID-19 during the trial to be 1%. An interim analysis is planned when 50% of the symptomatic COVID-19 cases are observed, and the overall type I errors will be controlled within 5% (both sided) by using O'Brien - Fleming spending function. In order to test that the vaccine protection effect is no less than 60% (95%CI lower bound > 30%), 155 cases of symptomatic COVID-19 and 22,144 subjects (11,072 subjects in each group) in total are required to achieve 90% power. The number of

events is calculated by using the exact condition method under the large sample poisson distribution hypothesis of Chan and Bohidar. Therefore, taking into account the dropout, protocol deviation, non-compliance, 14,000 subjects are planned to be recruited in each group.

Sample size calculation based on immunogenicity bridging study

Set a non-inferior effect value of 0.67, the inspection level α of 0.025 unilateral, and a master degree of 90%, and assume that the GMT of anti-SARS-COV-2 neutralizing antibody in people in China and outside China is the same, the standard deviation of the antibody titer after logarithmic conversion is 0.55, and the distribution ratio of the two groups of samples is 1:1. Using PASS 15, the minimum sample size of each Investigational product group in China and outside China was 207. Considering factors such as shedding and age distribution, 1000 subjects were planned to be enrolled in each experimental vaccine group in China and outside China (750 subjects aged 18-59 and 250 subjects aged 60 and above).Therefore, a total of 20,000 patients are to be enrolled, including 1,000 in China and 1,000 outside China (1,000 subject outside China will be immunogenicity subgroup of the efficacy study cohort, which will also participate in the efficacy evaluation). There will be 1000 in the investigational vaccine group and 1000 in the placebo group.

To sum up, the total sample size of the Phase III clinical trial will be 29,000.

Because of the unpredictable number of cases in some countries and centers and the change in incidence rate in countries, the number of COVID-19 cases will be reviewed in blinded manner during the trial. The number of subjects planned to be enrolled could be increased during the trial according to the change in incidence rate of different regions, but the number of symptomatic COVID-19 cases required could not be changed, therefore, it will not cause inflation of the overall type I errors.

Suspension or termination criteria for the trial

In case that new data on the Investigational product in this study or any other study is available, and the regulatory authorities, sponsor, investigator, and/or institutional review board / independent ethics committee believe that the trial shoud be suspended/terminated;

Suspension criteria for the trial

In case of any of the following situations, the trial shall be suspended, and the institutional review board / independent ethics committee and relevant

regulatory authorities shall be notified immediately, and an emergency expert meeting shall be convened to conduct safety demonstration analysis to determine whether to continue the trial.

Events leading to trial suspension	**Number, %**
Grade 3 or above AEs that last 48 hours after any dose of vaccination	>20% of all vaccinated

Termination criteria for the trial

In case of any of the following situations, the trial shall be terminated, and the institutional review board / independent ethics committee and relevant

regulatory authorities shall be notified immediately.

Events leading to trial termination	**Number/%**
Grade 3 or above AEs that last 48 hours after any dose of vaccination	>30% of all vaccinated

Data and safety monitoring board (DSMB) Responsibility

Safety data monitoring:

A DSMB will be set up to monitor the safety data during the study.

(1) Verify, approve and complete relevant work in time according to the stipulations in chapters of DSMB;
(2) Verify study protocol, verify efficacy/safety data and raise suggestions for revision of monitoring plan;
(3) Execute the verification of data at unblind state according to the

	monitoring plan. The efficacy/safety data are exhibited at unblind state through the information of actual study grouping (including: true name of two groups). (4) If the conditions are allowed, the factors beyond study are explored, such as scientific or therapeutic progress possibly causing a problem in safety of subjects or ethics of study. (5) Participate in discussion of DSMB, and vote for suggestions of DSMB when necessary; (6) Suggest the sponsor for other modifications during the course of study and after the termination of study based on the observed data; (7) If severe, critical or fatal case occurr after the subject is infected with SARS-COV-2 during study period, a special investigation should be conducted. The DSMB shall conduct an analysis based on the findings of the specific investigation. If the analysis suggests that ADE/VED exists, the DSMB shall convene an emergency meeting to assess the risk of ADE/VED in the entire trial and immediately report it to the institutional review board (IRB) / independent ethics committee (IEC) and relevant regulatory authorities.
Endpoint adjudication committee (EAC) responsibility	1) Able to review, approve and complete related work in a timely manner in accordance with the EAC charter; 2) The chairman is responsible for supervising whether the review of the endpoint event is carried out in accordance with the trial protocol; shall attend all meetings; record (only all the review results) in the summary report form and sign; responsible for checking meeting minutes and signing; coordinate and reach a consensus, and communicate the views to the sponsor; 3) In case of any endpoint event, objectively evaluate the endpoint event according to the unified definition standards, combined with clinical expertise and relevant contents in the protocol to determine whether it conforms to the definition standards;

	4) Review the description of all events and check the source documents of each event. Necessary relevant information in the source documents obtained by the committee members have to be masked to ensure that blind review is achieved; 5) During the independent review process, the committee members can request to provide the required source documents, and review relevant clinical data (i.e., lung imaging, death certificate, hospitalization records, etc.) before making the final decision; 6) Members reach a consensus on the independent review, and the review results will be announced at the meeting and the chairman will sign for confirmation in the summary report form. If the independent review opinions of the committee members cannot reach a consensus, review meetings (regular meetings or ad hoc meetings) will be held as necessary to discuss them. If there are still disagreements after the discussion, voting shall be performed. Voting must also follow certain rules; 7) If the committee members need additional source documents during the review meeting, they should be recorded in the meeting minutes and make supplementary application after the meeting; 8) The EAC management team needs to cooperate with the data management department to complete the data question proposal and answer for the review results, which is different from the general clinical trial question answering process; 9) After completing evaluation, formulate the final Master Binder.
Case monitoring	After the first dose of vaccination, if the subjects have symptoms in consistent with suspected COVID-19, they must contact the investigators in timely manner, and the investigators will determine the suspected case according to the suspected case definition. Subjects who are determined to be suspected cases shall visit the study facility or designated institutions for collection of throat swabs from the subjects, and undergo SARS-COV-2 nucleic acid detection

	by real-time fluorescent quantitative RT-PCR.
Definitions	1) "Month" in the visit: defined as "30 days"; 2) Women of childbearing age: refers to women in a specific period from the development of female reproductive organs (menarche) to ovarian function decline (menopause); 3) 18-59 years of age: 18 years old or above on the day of enrollment (i.e. the day of 18 years old), but less than 60 years old (i.e. the day before 60 years old); 4) 60 years and above: at least 60 years old on the day of enrollment (i.e., 60 years old) or above. 5) COVID-19 diagnostic criteria: laboratory confirmed (SARS-COV-2 nucleic acid positive by real-time fluorescent quantitative RT-PCR) SARS-COV-2 infection; 6) COVID-19 of Any Severity: COVID-19 infection confirmed in laboratory, and having at least one of the following symptoms: fever (axillary temperature ≥37.3° C, oral temperature ≥ 37.5° C), cough, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell / taste. 7) Suspected COVID-19: Those who meet any of the following conditions: (1) having fever (axillary temperature ≥37.3° C/ oral temperature ≥37.5° C), cough and shortness of breath; (2) having chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell / taste for consecutive two days or above; (3) having the symptoms described in (2) but not lasting for two days or above, judged by investigators as suspected case based on their own clinical experience, inquiry of current medical history or epidemiological history and other information. 8) Mild COVID-19: Symptomatic patients with laboratory confirmed COVID-19infection and no evidence of viral

	pneumonia or hypoxia. Symptoms include fever, cough, fatigue, anorexia, shortness of breath, myalgia, sore throat, nasal congestion, headache, diarrhea, nausea, vomiting, loss of smell (anosmia), loss of taste (ageusia), etc; 9) Common COVID-19: Laboratory confirmed COVID-19 infection with clinical signs of pneumonia (fever, cough, dyspnea, shortness of breath) but no signs of severe pneumonia, including SPO2 ⩾ 90% under indoor conditions; 10) Severe COVID-19: COVID-19 infection confirmed in laboratory, and meet any of the following conditions:1) In case of shortness of breath, RR ⩾ 30 times / min; 2) under resting state, oxygen saturation ⩽ 93% during air inhalation; 3) arterial partial pressure of oxygen (PaO2) / oxygen inhalation concentration (FiO2) ⩽ 300 mmHg (1mmhg = 0.133kpa); PaO2 / FiO2 should be corrected according to the following formula: PaO2 / FiO2 X [760 / atmospheric pressure (mmHg)]; 4) the clinical symptoms worsened progressively, and the lung imaging showed that the lesions progressed more than 50% within 24-48 hours.; 11) Critical COVID-19: laboratory confirmed COVID-19 infection with acute respiratory distress syndrome, sepsis, septic shock, and other complications of COVID-19 patients including acute, life-threatening conditions such as acute pulmonary embolism, acute coronary syndrome, acute stroke, and delirium.
Duration of Study	Each subject will participate in the clinical trial for about 14 months.

2. Glossary of terms

Standard operating procedures	Standard and detailed written procedures to effectively implement and complete each task in one clinical trial.
Case report form	Paper or electronic document recording subject's relevant data reported to the sponsor that is designed as required in the study protocol.
Auditing	Systematic and independent inspection of clinical trial related activities and documents as to evaluate the implementation of clinical trial related activities, if the record, analysis and reporting of study data meet the study protocol, standard operating procedures and requirements in relevant laws and regulations.
Monitoring	One action to monitor the progress of clinical trial, and make sure the clinical trial is conducted, recorded and reported in accordance with the study protocol, standard operating procedures and requirements in relevant laws and regulations.
Investigational product	Vaccines for clinical trials, including investigational vaccine and placebo.
Randomization	The randomization principle in clinical trials is defined as the implementation process or measure in which each subject in the clinical trial has equal chance to be assigned to the study group or placebo group, the randomization process will not be affected by investigator's and/or subject's subjective will.
Blinding	Blinding is one of the important measures to control the bias resulted from awareness of randomized grouping information in clinical trials, and for the purpose of achieving the unpredictability of randomization by all the parties in clinical trials.
Subjects	Recipients of investigational vaccine who participate in one clinical trial, including patient, healthy subject.

Dropout	Defined as inability to receive the last follow-up required in the study protocol for any reason.
Investigator's Brochure	Clinical and non-clinical study data on the Investigational product available when conducting human trials.
Preventive drugs	The drugs for prevention of possible solicited AEs in the solicitation period after vaccination.

Informed consent form	Defined as the documentary evidence on each subject's willingness to participate in one trial.The property, objective, possible benefits and risks, the available other therapeutic options, rights and obligations for subjects in compliance with the Declaration of Helsinki need to be described by investigators to the subjects, allowing subjects to express their consent upon full awareness.
Adverse events	All the untoward medical events occurred in a subject after administration of an investigational vaccine, which do not necessarily have a causal relationship with the treatment.
Solicited adverse events	Adverse events collected as safety endpoints in the clinical study, defined as the data on the adverse events actively solicited by investigators or subjects within specific follow-up period after vaccination.
Unsolicited adverse events	Other adverse events except the solicited adverse events reported in clinical studies, also including the solicited adverse events reported beyond the designated time window of solicitation.
Serious adverse events	Death, being life-threatening, permanent or serious disability or loss of function, requiring hospitalization or prolonged hospital stay, as well as congenital anomaly or birth defects and other medical events after administration of Investigational product.
Data and safety monitoring committee	One independent committee comprised of professionals with relevant professional knowledge and experience, which is established by the sponsor for regular evaluation of the progress of clinical trial, safety data and key efficacy variables, and can give advice to the sponsor on continuation, modification or discontinuation of the trial.
Endpoint adjudication committee	One committee comprised of clinical experts who adjudicate the primary evaluation endpoints of the clinical trial according to standard working procedures. Regardless of the use of blinding in the clinical trial, the committee experts should be kept blind

	to the evaluated subject at the evaluation of endpoint.

3. Abbreviations

COVID-19	Coronavirus Disease 2019, a disease caused by SARS-COV-2 virus
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EAC	Endpoint Adjudication Committee
RBD	Receptor Binding Domain
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
PI	Principal Investigator
IA	Interim Analysis
AE	Adverse Event
SAE	Serious Adverse Event
ADE	Antibody Dependent Enhancement
VED	Vaccine Enhanced Diseases
Arthus	Local Anaphylactic Necrosis Reaction
CI	Confidence Interval
IRB	Institutional Review Board
NMPA	National Medical Products Administration
SPO_2	Blood Oxygen Saturation
WHO	World Health Organization
SARS-COV	Severe Acute Respiratory Syndrome Coronavirus
SARS-COV-	Severe Acute Respiratory Syndrome Coronavirus type 2, the virus

2	that causes COVID-19
SOP	Standard Operation Procedure
MCHC	Mean Corpuscular Hemoglobin Concentration
MPV	Mean Platelet Volume
RDW	Red Cell Distribution Width
#NEUT	Neutrophil Count
%NEUT	Neutrophil Percentage
#LYMPH	Lymphocyte Count
%LYMPH	Lymphocyte Percentage
#MONO	Monocyte Count
%MONO	Monocyte Percentage
#EOS	Eosinophil Count
%EOS	Eosinophil Percentage
#BASO	Basophil Count
%BASO	Basophil Percentage
#LUC	Large Unstained Cell Count
%LUC	Large Unstained Cell Percentage
#RETIC	Reticulocyte Count
%RETIC	Reticulocyte Percentage
PT	Prothrombin Time
APTT	Activated Partial Thrombin Time
Fbg	Fibrinogen
RBC	Red Blood Cell Count
WBC	White Blood Cell Count
HGB	Hemoglobin
PLT	Platelet Count

HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
PBLC	Peripheral Blood Lymphocytes
IL-4	Interleukin-4
IL-2	Interleukin-2
IFN-γ	Interferon-gamma
CTL	Cytotoxic T Lymphocyte
NOAEL	No Observed Adverse Effect Level
GMT	Geometric Mean Titer
RT-PCR	Reverse Transcription Polymerase Chain Reaction
MERSR-COV	Middle East Respiratory Syndrome Coronavirus
VERO E6	African Green Monkey Kidney Cell Line
HUH-7	Human Hepatoma Cells
BAT-SL-COVZC45	Bat SARS-Like Coronavirus
NCP-RBD	Novel Coronavirus Spike Protein - Receptor Binding Region
ELISpot	Enzyme Linked Immunospot Assay
GMP	Good Manufacturing Practices
RR	Respiratory Rate
ECMO	Extracorporeal Membrane Oxygenation
CHO Cell	Chinese Hamster Ovary Cell
eCRF	Electronic Case Report Form
PV	Pharmacovigilance

4. Background and Rationale

4.1. Disease and pathogen

4.1.1. Disease setting

Since December 2019, there have been many cases of pneumonia patients infected by the Novel Coronavirus in Wuhan, Hubei Province. With the spread of the epidemic, such diseases have also occurred in other regions of China and abroad. As of October 21st, 2020, the total number of confirmed cases worldwidehas reached 41 million, the total number of deaths worldwide is more than 1.12 million. On January 31, 2020, the World Health Organization declared the outbreak of the Novel Coronavirus as a global public health emergency. On February 11, 2020, the World Health Organization announced that the Novel Coronavirus-infected pneumonia official name"COVID-19". Subsequently, the World Virus Classification Committee named the virus as severe acute respiratory syndrome Coronavirus type 2 (SARS-COV-2).

At present, the source of infection with the Novel Coronavirus is mainly patients infected by the Novel Coronavirus, and asymptomatic infected patients may also become the source of infection. The main transmission routes are droplet transmission, contact transmission, fecal-oral transmission, and respiratory aerosol transmission of different sizes. Based on current epidemiological investigations, the incubation period of the disease is 1 to 14 days, mostly 3 to 7 days. The main symptoms of patients include fever, fatigue, and dry cough. A few patients are accompanied by nasal congestion, runny nose, sore throat and diarrhea. Severe patients often develop dyspnea and/or hypoxemia one week after the onset. In severe cases, the symptoms rapidly progress to acute respiratory distress syndrome, septic shock, difficult to correct metabolic acidosis, and coagulopathy. Judging from the status of the currently admitted cases, most patients have a good prognosis, and a few patients are in critical condition. The prognosis of the elderly and those with chronic underlying diseases is poor. Symptoms in children are relatively mild.[1]

4.1.2. Pathogen background

The Coronavirus particle diameter is between 70-120 nm and contains a single non-segmented RNA genome with a length of 26-32KB, encoding and duplicating enzyme protein, spikes protein (S protein), envelop small membrane protein (E protein), membrane protein (M protein) and nucleocapsid protein (N protein)[2] from 5 prime end to 3 prime end. Among these structural proteins, S protein mediates the

adhesion and entry of Coronavirus into host cells. The receptor-binding domain (RBD) is low in conservation among different viruses, and contains a variety of conformational neutralization tables. This allows it to easily spread across a wide range of hosts across cell and tissue types and even species barriers. The diversity of RBD also determines the types of host receptors used by different Coronaviruses and the way they enter host cells. SARS-COV binds to angiotensin-converting enzyme 2 (ACE2) receptors, and MERS-COV binds to two Dipeptidyl peptidase 4 (DPP4) receptor. The latest research shows that SARS-COV-2 may also enter human cells by binding to the ACE2 receptor. The above structural features provide a structural basis and theoretical basis for designing specific vaccines for different Coronaviruses.[2][3][4-5][6]

The Novel Coronavirus belongs to the beta genus of Coronaviruses. It has an envelope, and the particles are round or oval, often pleomorphic, with a diameter of 60-140nm. Its genetic characteristics are significantly different from SARS-COV (Severe Acute Respiratory Syndrome Coronavirus) and MERS-COV (Middle East Respiratory Syndrome Coronavirus). Current research shows that the homology with bat SARS-like Coronavirus (bat-SL-CoVZC45) is more than 85%. When isolated and cultured in vitro, SARS-COV-2 (Novel Coronavirus) can be found in human respiratory epithelial cells in about 96 hours, while it takes about 6 days in Vero E6 (African green monkey kidney cell line) and Huh-7 cells (human liver cancer cells). Most of the understanding of the physical and chemical properties of Coronavirus comes from the study of SARS-COV and MERS-COV. Viruses are sensitive to ultraviolet rays and heat. At 56°C for 30 minutes, 75% ethanol, chlorine-containing disinfectants, peracetic acid and chloroform and other lipid solvents can effectively inactivate the virus, but chlorhexidine cannot effectively inactivate the virus.[1]

4.2. Investigational product

4.2.1. Product characteristics

This vaccine is made by purified receptor binding region of the Novel Coronavirus spike glycoprotein (recombinant protein NCP-RBD) expressed by recombinant CHO cells and adding aluminum hydroxide adjuvant. It is used to prevent respiratory diseases caused by Novel Coronavirus infection. It is a milky white suspension liquid, which can be stratified due to precipitation and after shaking, it should be easy to disperse.

4.2.2. Product specification

The company's quality standards for the Recombinant Novel Coronavirus Vaccine (CHO cells) was formulated according to the research data of quality and registration standards, along with the *"Regulations for the Administration of Drug Registration"*, the *"Registration Classification and Application Data Project Requirements for Biological Products"*, ICH guidelines, and *"Technical Guidelines for Quality Control of Human Recombinant DNA Products"* , *"Chinese Pharmacopoeia"* (2015 edition), see the table below.

Table 4 Quality Standards of Recombinant Novel Coronavirus Vaccine (CHO Cell)

No	Test items	Quality control standards
1	Identification test	Coronavirus NCP-RBD antigen protein should be detected by enzyme-linked immunosorbent assay (ELISA).
2	Appearance	It should be a milky white suspension liquid, which can be stratified due to precipitation. After shaking, it should be easy to disperse, and there should be no lumps.
3	Quantity	According to law (General Rule 0102), it should not be less than the marked quantity.
4	Osmolality	280mOsmol/kg±65mOsmol/kg（General Rule 0632）
5	pH	5.0~7.0（General Rule 0631）
6	Aluminum content	0.35~0.65mg/ml（General Rule 3106）
7	Efficacy test	Vaccinate 10 female Balb/c mice of 4 to 8 weeks of age with test samples of each specification. Each mouse is injected intraperitoneally with 0.5ml test product. The vaccine reference product is used as a parallel control, and physiological saline is used as a negative control. After 2 weeks, blood is collected, and the level of anti-NCP-RBD antibody is detected by enzyme-linked immunosorbent assay, and the GMT value is calculated. The GMT ratio of the test product/vaccine reference product with a specification of 50μg/dose should be no less than 0.4, and the GMT ratio of the test product with a specification of 25μg/dose should be no less than 0.4. The GMT ratio of the test product/vaccine reference product should be no less than 0.2.
8	Sterility test	According to the rule（General Rule 1101），should be in compliance..
9	Abnormal toxicity test	According to the rule（General Rule 1141），should be in compliance..
10	Bacterial endotoxin test	Should be less than 10EU/ml（General Rule 1143 Gel

No	Test items	Quality control standards
		limit test method）

4.2.3. Stability studies

The company's plan for studying the stability of the Recombinant Novel Coronavirus Vaccine (CHO cell) bulk was formulated according to the *"New Drug Approval Regulations* ", "*Chinese Pharmacopoeia"* (2015 Edition) and the *"Technical Guidelines for Stability Research of Biological Products".*

The research of the stability of the bulk solution mainly includes: long-term stability study (2~8℃/-20±2℃ storage for 18 months), accelerated stability test (25 ±2℃) and mandatory conditions (repeated freezing and thawing, high temperature, vibration) test. During the entire inspection process, samples were taken at each time point, and tested according to different test items. The basic data can be used for reference in production and storage during scale-up production. The samples investigated in this study are shown in the table below.

Table 5 Conditions, research indicators and research results of vaccine stability studies

Types of tests		Environmental conditions	Planned sampling time point	Test items
Mandatory condition test	Shaking test	2~8℃, shaking	7、14、21、28 day	On the 7th, 14th and 21st day, test appearance, pH value, bacterial endotoxin and efficacy.
	Illumination test	2~8℃, Light intensity 4500lx±500lx	7、14、21、28 day	
	High temperature test	37±2℃	7、14、21、28 day	
Accelerated stability		25±2℃	1、2、3、6 month	Test appearance, pH, bacterial endotoxin and efficacy in the 1st month, test appearance, pH, bacterial endotoxin in the 2nd and 3rd month, test appearance, pH, bacterial endotoxin, sterility inspection, and efficacy in the 6th month.

Long-term stability	2~8℃	0、3、6、9、12、18、24、30 month	Only test the appearance, bacterial endotoxin and pH value in the 3rd, 9thand 18thmonth, and full inspections will be conducted at 0, 6th, 12th, 24th and 30th month.

At present, the research of mandatory condition stability test, accelerated stability test and long-term stability test of the vaccine is still in progress. All tests arecompleted and within the qualified rangeStability tests under mandatory conditions of the high temperature, shaking, and illumination have been monitored for 28 days, and the appearance, pH, bacterial endotoxin and efficacy tests have been completed in compliance with the regulations.

The accelerated stability testhas been completed observation for 6 months The appearance, pH, efficacy and bacterial endotoxin tests have been completed, all the samples are in compliance with regulations. The long term stability test also has been completed the observation for 6 months, and all results of the tests are in compliance with regulations.

4.2.4. Storage conditions

The vaccine should be stored under the temperature of 2 ~ 8 ℃, away from light and strictly prevent freezing. Temperature should be monitored and recorded daily during storage.

4.3. Nonclinical studies of the vaccine

4.3.1. Pharmacology studies

4.3.1.1. Primary pharmacology

4.3.1.1.1. Immunogenicity analysis in mice

(1) Binding antibody level result

In this study, Balb/c mice were immunized with this vaccine (immunization schedule is 0D-14D). 14 days after the second immunization, blood was collected and sera was separated for detection of NCP-RBD antigen-specific Binding antibody levels.The GMT value of antibody titer can reach as high as $10^{5.}$

The results showed that the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of high level of antigen-specific Binding antibody titer in mice after immunizing, indicating that the vaccine has good immunogenicity.

(2) Live virus neutralizing antibody level detection

The sera of the mice immunized with two injections of the above vaccine were tested by the Institute of Microbiology, Chinese Academy of Sciences for the level of live virus neutralizing antibodies. The GMT value of neutralizing antibody titer was 228.1

The results showed that after immunization in mice, a strong neutralizing affect is detected by authentic virus neutralizing antibody test, which proves that the vaccine has good immunogenicity.

4.3.1.1.2. Immunogenicity analysis in rats

In this study, rats were immunized intramuscularly with the vaccine and the cellular and humoral immune responses in rats were studied. The SD rats, SPF grade, 120 in total, half male and half female (age before administration: 7-9 weeks old, weight before administration: male 260-330g, female 179-234g) were divided into 4 groups by weight balancing randomized grouping method. There were 30 animals in each group, which were the blank control group, adjuvant control group, low-dose group and high-dose group. The four groups were given intramuscularly with 0.5 mL of 0.9% sodium chloride injection/ animal, placebo (including aluminum adjuvant) 0.5mL/animal, 0.25 mL vaccine (1/2 human dose)/animal, 0.5mL vaccine (1 human dose)/animal respectively. The animals were administered 3 times on D1, D15, and D29, and after full course of administration, the animals were observed for 2 weeks (recovery period).

(1) Binding antibody level result

The rat immunogenicity study of the Recombinant Novel Coronavirus Vaccine (CHO cells) was carried out along with repeated dose toxicity test in rats for 4 weeks. After three doses of immunization, blood was collected for GMT (NCP-RBD) testing during the withdrawal and recovery period.

The results showed that the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of antigen-specific IgG antibodies in the SD rats after immunization. After the third immunization, there was no significant difference in GMT (NCP-RBD) between the high and low dose groups during the withdrawal examination and recovery period, and there was no significant difference in GMT (NCP-RBD) between the different dose groups during the withdrawal and recovery period. However, the GMT (NCP-RBD) value of binding antibody level in both the

high and low dose groups reached 10^6, indicating that the vaccine could induce the production of high titer Binding antibody after immunization in rats, showing good immunogenicity.

(2) Pseudovirus neutralizing antibody test result

The rat immunogenicity study of Recombinant Novel Coronavirus Vaccine (CHO cell) was carried out with repeated administration toxicity test in rats for 4 weeks. After third immunizations, the sera of female rats during the withdrawal period was tested for the level of pseudovirus neutralizing antibodies.

The results showed that this vaccine can induce the secretion of antigen-specific Binding antibody and pseudovirus-neutralizing antibody in rats (SD). After the third immunization, Binding antibody the antibody titer of pseudovirus-neutralizing antibody can reach 10^3, which verifies the good immunogenicity of this vaccine.

4.3.1.1.3. Immunogenicity analysis in crab-eating macaques

This study was carried out along with repeated dose toxicity test in monkeys for 10 weeks (0-4w-8w-10w). Crab-eating macaques were immunized intramuscularly to study the effect of the vaccine in inducing cellular and humoral immune responses in primates. 40 general-grade crab-eating macaques qualified for quarantine were selected, half male and half female (age before administration: 3 to 4 years old, male weight 2.95 to 3.67 kg, female weight 2.97 to 3.62 kg) and randomly divided into 4 groups, namely blank control group, adjuvant control group, low-dose group, high-dose group, with 10 animals in each group (♀♂ half each). The blank control group was injected intramuscularly with 0.9% sodium chloride injection 1.0 mL/animal, and the adjuvant control group was injected intramuscularly with 1.0 mL vaccine placebo (containing aluminum adjuvant) per each animal. The low and high dose groups were injected intramuscularly 0.5mL (1 human dose)/animal and 1.0mL (2 human doses)/animal, for a total of 4 administrations (at week 0, week 4, week 8, week 10), after full course of administration, the animals were observed for 2 weeks (recovery period).

(1) Binding antibody level result

Crab-eating macaques were immunized with the vaccine according to the procedure of week 0-week 4-week 8-week 10. Blood was collected before the first dose, before the second dose, before the third dose, before the last dose and at the end

of the recovery period.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of antigen-specific IgG antibodies in Crab-eating macaques. As the number of immunization times increases, the corresponding GMT increases accordingly. After the second immunization, the antibody level can reach the level of 10^7. It shows that the vaccine has good immunogenicity on crab-eating macaques; there is no significant difference in the level of antibodies produced by the high-dose group and the low-dose group during different immunization doses, indicating that different immunization dosage have no significant impact on the production of antibodies; For both the high-dose group and the low-dose group, there was a significant difference between the second dose and the third dose, and there was no significant difference between the antibody levels before the third dose, before the last dose, and during the recovery period. Therefore, the number of immunizations for this vaccine should be no less than two doses.

(2) Pseudovirus neutralizing antibody test result

In this study, blood samples of the crab-eating macaques were collected before the first immunization, before the second immunization, before the third immunization, before the last immunization, and during the recovery period to test the levels of pseudovirus neutralizing antibodies.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of neutralizing antibodies in crab-eating macaques. As the number of immunization times increases, the corresponding pseudovirus neutralizing antibodies increase accordingly. After the second immunization, the antibody level can reach 10^4, indicating that the vaccine humoral immunity induced by the vaccine in crab-eating monkeys have potential high neutralization effect on virus;; for the high-dose group, there is a certain difference before the third immunization and before the last immunization, but there is no significant difference between the third immunization and the recovery period; In the low-dose group, there was a significant difference before the third immunization and before the last immunization, and there was no significant difference before the last immunization and the recovery period; for the high-dose group and the low-dose group, before the third dose, there was a significant difference in the levels of pseudovirus neutralizing antibodies (*$P<0.05$), and there was no significant difference in the levels of pseudovirus neutralizing

antibodies before the last administration and during the recovery period.

(3) Live virus neutralizing antibody test result

In this study, blood sample of the crab-eating macaques were collected before the third immunization and before the last immunization, and the level of authentic virus neutralizing antibodies was tested.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of neutralizing antibodies against the authentic virus in crab-eating macaques, and after the second immunization, the level of neutralizing antibody reached 10^3, indicating that the humoral immunity induced by the vaccine in crab-eating monkeys can effectively neutralize the live virus. There was no significant difference in neutralizing antibody levels among the high-dose group and the low-dose group and there was no significant difference between the high-dose group and the low-dose group before the third dose and before the last dose. After the second immunization, the level of neutralizing antibody reached 10^3.Therefore, there is no significant difference in authentic virus neutralizing antibody level among crab-eating macaques administered with different dosages.

(4) Cellular immune test results

The IL-2 ELISPOT, IFN-γ ELISPOT and IL-4 ELISPOT tests were carried out during the drug withdrawal and recovery period in the 10-week repeated dose toxicity test of the Recombinant Novel Coronavirus Vaccine (CHO cells) in monkeys (i.m. injection). The results are as follows:

1) IL-2 ELISPOT test results: the spleen lymphocytes in both high and low dose groups produced effective and strong cellular responses against the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, *P<0.05), indicating that the antigen could effectively stimulate the secretion of IL-2 cytokines by spleen lymphocytes.

2) IFN-γ ELISPOT test results: The spleen lymphocytes in both high and low dose groups produced effective and strong cellular responses against the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, *P<0.05), indicating that the antigen could effectively stimulate the secretion of IFN- cytokines by spleen lymphocytes.

3) IL-4 ELISPOT test results: The spleen lymphocytes in both high and low dose

groups produced effective and strong cellular responses to the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, **P<0.01), indicating that the antigen could stimulate the secretion of IL-4 cytokines by spleen lymphocytes.

In summary, the results of the antigen-specific IL-2 ELISpot, IFN-γ ELISpot, and IL-4 ELISpot experiments of Crab-eating macaques during the drug withdrawal period and the end of the recovery period show that after injection of the Recombinant Novel Coronavirus Vaccine (CHO cells) in crab-eating macaques, the spleen lymphocytes of the high and low dose groups had a strong cellular response to the NCP-RBD antigen which is of significant difference comparing to the adjuvant control group and the blank control group, indicating the vaccine has good cellular immune effectin primates.

4.3.1.1.4. Pharmacology study conclusions

In summary, similar to the results of studies in mice and rats, this vaccine can also efficiently induce humoral immune responses in crab-eating macaques. The detection and evaluation of antibodies in animal sera show that immunization of different animals with this vaccine can induce the body to produce high levels of neutralizing antibodies. The results of the antigen-specific IL-2 ELISpot, IFN-γ ELISpot, and IL-4 ELISpot experiments of the Crab-eating macaques monkeys during the drug withdrawal inspection period and the end of the recovery period show that the vaccine can effectively stimulate specific cellular immune responses in the Crab-eating macaques after immunization. All results indicate that the vaccine has good immunogenicity and can induce a wide range of humoral and cellular immune responses.

4.3.1.2. Challenge test

Animal protection research is an important part of evaluating the immune effect of vaccines. Through the study of Coronavirus challenge on the Recombinant Novel Coronavirus Vaccine (CHO cells) ACE2 transgenic mice, to evaluate the anti-infection effect of ACE2 transgenic mice after immunization. Mice were immunized with one injection on day 0 and day 21, 0.1ml/mouse (containing 10μg of antigen), and challenged 7 days after the two doses, the challenge dose was 5×10^5 TCID50/mouse.

Experiment conclusions: 1) After the control group was challenged, the body

weight decreased significantly in the first 3 days. The virus titer of lung tissue reached $10^{5.32}$-$10^{7.99}$ TCID50 per gram. The lung lesions are obvious, with severe interstitial pneumonia. 2) After the immunization group was challenged, the weight loss was slight in the first 3 days. The virus titer of lung tissue reached 10^{2}-$10^{3.8}$ TCID50 per gram, a decrease of $10^{1.52}$-$10^{5.99}$ compared with the control group, and an average decrease of 3.6 Log times (3981 times). Lung lesions are mild, with mild interstitial pneumonia. It suggests that vaccine immunity has obvious protective effect.

4.3.1.3. Safety Pharmacology Studies

Crab-eating macaques were given 0.9% sodium chloride injection 1.0 mL/mouse (blank control group), Recombinant Novel Coronavirus Vaccine (CHO cells) placebo 1.0 mL/mouse (adjuvant control group), Recombinant Novel Coronavirus Vaccine (CHO cells) 0.5mL/mouse (low-dose group) and 1.0mL/mouse (high-dose group) have no significant effect on the ECG, blood pressure, respiration, body temperature and other indicators of Crab-eating macaques. The ECG, blood pressure, respiration, body temperature and other indicators of the adjuvant control group, low-dose group, and high-dose group were basically similar to those of the blank control group at the corresponding time ($P>0.05$). The fluctuations of blood pressure, respiration, body temperature and other indicators are caused by normal stress changes or circadian rhythms of monkeys, and have nothing to do with the test product.

The blank control group, adjuvant control group, low-dose group, and high-dose group showed consistent changes in the ECG, blood pressure, respiration, and body temperature indicators, that is, the change trend of each index is similar in the time period of -1h to 5h after administration, 5-6h after administration, and 6-24h after administration, and there was basically no difference among groups. The fluctuations of blood pressure, respiration, body temperature and other indicators are caused by normal stress changes or circadian rhythms of monkeys, and have nothing to do with the test product. It is comprehensively judged that the NOAEL of the Recombinant Novel Coronavirus Vaccine (CHO cells) on the cardiovascular and respiratory systems of Crab-eating macaques is greater than 1.0 mL (2 servings) per mouse.

Therefore, under the conditions of this test dose, crab-eating macaques were given a single intramuscular injection of the Recombinant Novel Coronavirus Vaccine (CHO cell) developed by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. with a batch number of 202004003 to affect the cardiovascular and respiratory systems of Crab-

eating macaques. There is no obvious effect, and the NOAEL of cardiovascular and respiratory system safety pharmacology is greater than 1.0mL (2 servings, 100μg NCP-RBD)/head.

4.3.2. Toxicology studies

4.3.2.1. Single-dose study

4.3.2.1.1. Single dose toxicity study in Sprague Dawley (SD) rats

Rat single-dose toxicity test: select SD rats, half of the male and female, 10/sex/group, intramuscular injection on hind limbs, single dose; maximum dose or maximum tolerated dose, set according to the results of the preliminary test. 3 groups: solvent control group, adjuvant control group, vaccine group (2 human dose). Clinical observation, body weight; gross anatomy 14 days after immunization (if abnormal, conduct pathological tissue examination), make pathological section of injection site.

Under different dose conditions of this test, rats were injected intramuscularly with Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) 2 human dose /animal and Recombinant Novel Coronavirus Vaccine (CHO cells) placebo (containing aluminum) (batch number: 202003002) (adjuvant reference substance) 2 human dose /animal, nodule at the injection site appeared after the immunization of the rat, and no abnormal reaction was seen in the test, nor did it cause the death of the rats; there was no significant effect on the weight gain of the rat.

Pathological examination results showed that all rats in the immunization group and the adjuvant control group had obvious inflammatory reactions, adjuvant deposition foci, and lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. The pathological changes in the two groups were basically consistent, considering the typical reaction at the site of immunization of aluminum-containing adjuvants given by injection, no abnormal lesions were seen in other tissues and organs, and no toxic target organs were clearly shown.

Based on the above test results, the maximum dose of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) for a single intramuscular injection in rats is 2 human dose /animal, and the maximum tolerable dose >2 human dose /animal

4.3.2.2. Repeated dose studies

4.3.2.2.1. 4-week repeated dose toxicity study in SD rats

120 adult (10~12w) SD rats, 15 rats/type/group were tested. Intramuscular injection 1 dose/week, 3 times (N+1), namely injection at 0W, 2W, 4W; 4 groups: Solvent control group, adjuvant control group, low-dose group (0.5 human dose), high-dose group (1 human dose).

Observation indicators are: (1) routine indicators: clinical observation: once a day; weight, food intake: once a week; clinical examination (hematology, blood coagulation, blood biochemistry, electrolyte, bone marrow smear), gross examination (Including the weighing of main organs) and a full set of histopathological examinations: stop medication and once at the end of the recovery period. (2) Immunological index inspection: lymphocyte immunophenotype analysis, sera immunoglobulin (IgG), complement (C3, C4), once vaccine withdrawal and recovery period; (3) Antibody test: At the end of the withdrawal period and recovery period, blood samples were collected once for detection of antibodies (IgG) and neutralizing antibodies titers.

Under the dosage conditions of this test, rats were given intramuscular injections of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) 0.25 mL (0.5 human dose) for 4 consecutive weeks (total immunization 3 times, 1 time/2 weeks) only, 0.5 mL (1 human dose) per animal, and Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (containing aluminum) (batch number: 202003002) 0.5 mL per animal, mainly as follows: adjuvant control group, high-dose group. After the immunization of the rats, the nodules can be touched at the injection site (without recovery after 2 weeks after stopping the vaccine), the rest has no obvious abnormal reaction and no animal death; no obvious abnormal effect on the weight gain and food intake of the rats; During drug inspection, it was found that there were no obvious abnormalities in the urine and ophthalmological indicators of each group; hematological indicators can be seen in all the three groups. MPV increased and PT decreased; biochemical indicators can be seen in all the three groups. Na^+ and Cl^- increased in the dose group, GLO increased and A/G decreased in the high-dose group.

In the withdrawal period, the immunotoxicity index examination showed that the low and high dose groups of IgG, C3 and the number of spleen systems of male rats were correlated with increased doses, and the immune phenotype of peripheral blood lymphocytes was not abnormal; immunogenicity tests showed low and high dose

groups vaccine can induce high-titer anti-NCP-RBD IgG antibodies with good immunogenicity in rats.

The results of histopathological examination showed that all rats in all the three groups showed obvious inflammatory reactions, adjuvant deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. The degree of change of the adjuvant control group is similar to the high-dose group, and the low-dose group is slightly less severe. Considering the typical reaction of intramuscular injection of aluminum-containing adjuvant at the injection site, there were no abnormal lesions in other tissues. The necropsy at the end of the recovery period generally showed that the local effects of the above-mentioned immunization showed a certain recovery trend.

In summary, rats have been injected intramuscularly (3 times in total) of Recombinant Novel Coronavirus Vaccine (CHO cells) for 4 consecutive weeks, 0.25 mL (0.5 human dose) per animal, 0.5 mL (1 human dose) per animal. It caused nodules at the injection site, some blood biochemical index effects (MPV immunization, PTV, Na^+↑, Cl^-↑), inflammatory reaction at the injection site, adjuvant deposition foci, and lymph nodes at the injection site similar to the adjuvant control group Hyperplasia and lymphatic sinus phagocytosis, the above abnormalities may be related to vaccination containing aluminum hydroxide adjuvant vaccine; it may also cause immune-related indicators (GLO drugs, A/G drugs, IgG drugs, C3G, spleen system number ↑). It is related to the immune response after vaccination. Excluding the above effects, no obvious toxic effects were seen during the drug withdrawal inspection stage, and the toxic target organs were not clearly displayed. The inspection results will be further analyzed after the recovery period.

4.3.2.2.2. 4-week repeated dose toxicity dose ranging study in monkeys

Choose crab-eating macaques, half of the male and female, 4/sex/group, 8 in total. Set up 2 groups: (1) adjuvant control group (2) vaccine group (1 human dose). Intramuscular injection on hind limbs, a total of 3 immunizations (N+1), namely 0w, 4w, and 8w, once each.

During the whole trial period, clinical observation (once a day); weight, food intake (once a week); ECG, body temperature, clinical tests (blood routine, blood coagulation, blood biochemistry, ophthalmology, urine, etc.): immunization Before, after the second injection, 3D (interim immunization check), 3d after the last injection

(drug discontinuation check), 1 time; urine check 1 time before autopsy. 3 days after the last injection (the end of administration), anatomy was performed according to routine requirements, and the main organs were weighed, bone marrow smear (smear preparation), gross and pathological examinations were performed.

This part of the toxicology study was commissioned by the Safety Evaluation Research Center of Zhejiang Academy of Medical Sciences. The monkey intramuscular injection of Recombinant Novel Coronavirus Vaccine (CHO cells) was repeated for 4 weeks and the toxicity test has been completed for 0w and 2w.

In summary, under the dosage conditions of this test, the Recombinant Novel Coronavirus Vaccine (CHO cell) with batch number 202004003 provided by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. was subjected to 3 consecutive injections (D1, D15, D29). After injection, it has strong immunogenicity to crab-eating macaques, which can elicit strong cellular immune response and high titers of IgG antibodies; except for local effects of injection, no obvious toxic effects and toxic target organs are seen. No obvious systemic adverse reactions were observed at the dose of 0.5mL/animal (1 human dose).

4.3.2.2.3. 10-week repeated dose toxicity study in monkeys

Choose crab-eating macaques, ♀♂ half each, 5/sex/group, a total of 40. Set up 4 groups: (1) solvent control group (2) adjuvant control group (3) low-dose group (1 human dose) (4) high-dose group (2 human dose). Intramuscular injection on hind limbs, a total of 3 injection s (N+1), namely 0w, 4w, 8w, 10w once each. The recovery period is 2w.

During the entire test period, clinical observations were carried out every day (1 time a day during the adaptation period and recovery period, and 1 time each morning and afternoon during the administration period). After the injection, the local reaction of the animal administration site was observed (before each injection), observe once each at ~0.5h, ~1h, ~2h and ~6h after injection). Body weight and food intake are measured once a week; body temperature adaptation period is checked twice, each time before dosing, 4-6h after dosing, the next day and the third day of dosing. Electrocardiogram, ophthalmology, and clinical examination (blood routine, blood coagulation, blood biochemistry) were checked twice during the adaptation period, and once each at the end of the drug withdrawal check and the recovery period; and

once each of the urine routine withdrawal check and the end of the recovery period. PBLC immunophenotype, immunoglobulin (IgG, IgM), and complement (C3, C4) are checked once before administration, once drug withdrawal, and after the recovery period; specific anti-drug antibodies and neutralizing antibodies are checked each time before stopping the vaccine (3 days after the last dose) and at the end of the recovery period (15 days after the last dose), check once each. After the last injection 3d (drug withdrawal inspection), 15d (end of recovery period) according to the routine requirements, the main organs were weighed, bone marrow smear (smear preparation), gross and pathological examination, and spleen lymph was used cells were tested for CTL activity.

Crab-eating macaques were injected intramuscularly with the Recombinant Novel Coronavirus Vaccine (CHO cells) of batch number 202004003 for 10 weeks (4 times in total, 1 time each for 0w, 4w, 8w, and 10w) 0.5mL (1 human dose)/monkey, 1.0mL (2 human dose)/monkey, the Recombinant Novel Coronavirus Vaccine (CHO cell) placebo with batch number 202003002 (containing aluminum) 1.0mL/monkey, there is no obvious abnormal reaction in general clinical observation and local injection site, and no death ; No obvious abnormal changes in body weight, food intake, body temperature, blood biochemistry, electrocardiogram, urine, ophthalmology, organ weight and coefficient; drug withdrawal inspection, adjuvant control group, low-dose group and high-dose group %NEUT (Percentage of neutrophils) and #NEUT (number of neutrophils) increased, %LYMPH (percentage of lymphocytes) and #LYMPH (number of lymphocytes) decreased, and %EOS (percentage of eosinophils) and # in the high-dose group The increase in EOS (eosinophil count) reflects the appearance of typical inflammatory leukocyte blood picture after local intramuscular injection of the test product and adjuvant reference product containing aluminum adjuvant. The increase in EOS is also the performance of protein vaccine immunity, however, at the end of recovery period (2 weeks after drug withdrawal), the above abnormalities were down-regulated or up-regulated and returned to the reference range.

The results of histopathological examination showed that the pathological changes of the monkeys in the adjuvant control group and the low and high dose groups were mainly at the injection site and its lymph nodes. Examination at the end of the recovery period showed that all monkeys had obvious inflammatory reactions

and adjuvants at the injection site. Deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. At the end of the recovery period, the above changes showed certain signs and trends for recovery; the above three groups of test monkeys were mostly consistent in the degree of lesions at the drug administration site. The high-dose group had lesions of relatively severe degree, the adjuvant control group and the low-dose group have relatively mild lesions. The main consideration is the typical reaction of intramuscular injection of aluminum-containing adjuvant at the administration site; no other tissues and organs were found in the two periods. Product-related abnormal pathological changes.

The immunogenicity results showed that both the low and high dose could produce strong humoral immune responses (producing high titers of antigen-specific IgG antibodies, pseudovirus and true virus neutralizing antibodies) and cellular immune responses (specific Sex antigen stimulates the secretion of IL-2, IFN-γ and IL-4 of splenic lymphocytes to increase significantly) in crab-eating macaques, and has good immunogenicity. This product has immunotoxicity indicators for immunoglobulin (IgA, IgG), complement (C3, C4), peripheral blood PBLC immunophenotype, immune-related organ weight and coefficient, macroscopic and histopathological indicators of immune-related organs No obvious impact was seen.

To sum up, crab-eating macaques were injected intramuscularly with 0.5 mL (1 human dose) of Recombinant Novel Coronavirus Vaccine (CHO cells) for 10 consecutive weeks (4 times in total, once each for 0w, 4w, 8w, and 10w). (2 human dose)/animal, it can mainly cause inflammatory reaction at the injection site and adjuvant deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site similar to the adjuvant control group (there is a tendency to recover 15 days after stopping the drug), NEUT increased and LYMPH decreased. The above-mentioned abnormalities may be mainly related to vaccination of aluminum-containing adjuvant vaccines; it may also cause an increase in EOS, which may be related to the immune response of the vaccine. Excluding the above effects, under the dose conditions of this test, crab-eating macaques were injected intramuscularly with Recombinant Novel Coronavirus Vaccine (CHO cells) for 10 consecutive weeks (4 times in total). No obvious systemic and immunotoxicity effects were seen, and the target organs of toxicity were not obvious. It showed that the NOAEL dose was 1.0 mL (2 human dose, NCP-RBD protein 100μg)/head.

4.3.2.3. Other toxicity studies

4.3.2.3.1. Local injection site reactions

Twelve New Zealand rabbits, were randomly divided into 3 groups, 4 rabbits in each group, half male and half female, and quadriceps femoris were injected with a single injection of Recombinant Novel Coronavirus Vaccine (CHO cells), adjuvant control and solvent control group (left and right) 0.5ml on each side), 1/2 animals in each group were anesthetized and executed 48 hours after administration, and the quadriceps femoris changes at the injection site were dissected to observe the changes in the quadriceps muscle at the injection site, scored according to the muscle stimulation response grading standard, and histopathological examination was performed. Leave 1/2 animals in each group to continue to observe until 14 days after the administration and then undergo histopathological examination to understand the reversibility of the irritation response.

According to the naked eye and histopathological examination, the local pathological changes in the quadriceps femoris muscle of one rabbit in the negative control group after 48 hours of drug withdrawal were considered to be caused by mechanical damage to the injection operation; the placebo group and the test product group are stopped For 48 hours, the experimental rabbits were examined for focal adjuvant deposition and acute inflammatory reaction at the injection site. At the end of the recovery period, the interstitial chronic inflammatory reaction and obvious adjuvant deposition at the injection site were examined. Both were administered with the injection of aluminum adjuvant. The typical local reactions are the same, so the above changes are considered to be the typical local reactions of intramuscular injection of aluminum-containing adjuvants.

In the placebo group and the test product group, the local changes of the experimental rabbits were mostly interstitial changes. The necrosis of muscle fibers on one side of the placebo group was checked for 48 hours after the drug was stopped. It may be due to individual differences or mechanical damage and adjuvant deposition during injection The reaction is caused by the combined effect, and the muscle fiber atrophy at the end of the recovery period is considered to be caused by the local deposition of adjuvant. In general, the substantial irritant damage of the muscle fibers at the administration site of the placebo group and the test product group was not obvious, and was classified as "minor" according to the "Irritation Grade Judgment

Standard".

Under the dosage conditions of this test, according to the average score of muscle stimulation response, according to the "Irritation Grade Judgment Standard", the Recombinant Novel Coronavirus Vaccine (CHO cell) (batch number: 202004003) showed a slight irritation response to rabbit muscles, which is compatible with Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (containing aluminum) (batch number: 202003002) had basically the same response; since the dose of aluminum hydroxide adjuvant in this trial was 1.0 mg/rabbit, the dose was twice the human dose, so after 14 days of the recovery period (another 1/2 rabbits continued to observe for 14 days after the necropsy was stopped 48 hours), the irritation lesions at the administration site were not significantly repaired.

4.3.2.3.2. Animal allergy testing

Forty Guinea pigs, male and female, sensitized by intramuscular injection, once every other day, 3 times in total; intravenous injection of 2 times the sensitizing dose to challenge; set up 5 groups: negative control group, positive control group, adjuvant control group (1 human dose), low-dose group (0.2 human dose), high-dose group (1 human dose), observe the systemic allergic reaction after challenge.

Under the dosage conditions of this test, the high-dose group of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) had a certain active systemic allergic reaction to guinea pigs, which basically disappeared within 7 minutes after injection, and there was no death of guinea pigs; There was no active allergic reaction in the dose group and the negative control group; the Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (batch number: 202003002) had no active allergic reaction to guinea pigs.

For the high-dose group, the guinea pig's immunization dose was 1 human immunization dose, and the low-dose group was 0.2 human immunization dose. For this allergic reaction, the high-dose group had a certain active systemic allergic reaction, while for the low-dose group, no active anaphylaxis was seen, so it can be judged that the active anaphylaxis in the high-dose group is an allergic reaction caused by excessive immune adjuvant aluminum hydroxide.

4.4. Previous clinical studies results

4.4.1. Recombinant Novel Coronavirus Vaccine (CHO cell) Phase I clinical trial

The Phase I clinical trial of the Recombinant Novel Coronavirus Vaccine (CHO cell) in healthy people aged 18 to 59 years old is a multicenter, double-blind, randomized, placebo-control, dose escalation study. The investigational vaccine groups include a low dose group (25 μg/ 0.5ml/vial) and a high dose group (50μg/ 0.5 ml/vial).

A total number of 50 (20 in the low-dose vaccine group; 20 in the high-dose vaccine group; and 10 in the placebo group) subjects were enrolled. Subjects received one dose of the investigational vaccine or placebo at 0, 1, and 2 months respectively. At the first Phase of the study，25 subjects were randomly assigned to either the low-dose group (20 cases) or the placebo group (5 cases). 7 days after the subjects from the first Phase received the first dose, the DSMB conducted safety assessment, confirmed the safety and agreed to continue the trial and then the second Phase of the study was started and another 25 subjects were randomly assigned to either the high-dose group (20 cases) or the placebo group (5 cases). The safety of each subject is observed from the first dose of vaccination to 1 year after full vaccination so as to evaluate the safety and tolerability of the vaccine, during which blood samples are taken several times to preliminarily explore the immunogenicity of the vaccine.

The database of Phase I trial was locked and analyzed on October 22, 2020. The scope of database lock-in included the visit data and partial immunogenicity data collected by EDC before October 21, 2020.

The preliminary safety results of this study show that there is no significant difference between the high-dose group, low-dose group and placebo group in the overall incidence of adverse events and the incidence of adverse events related to the Investigational product among the groups. It can be seen that there is no significant difference in the incidence of adverse events between the investigational vaccine with different immunization dosages and the placebo. The incidence of adverse reactions at the site (local) of high and low dose vaccine is higher than that of placebo, but there is no statistical significance among the groups. After injection, the systemic and local reactions were mostly mild, transient, and the severity was mainly grade 1 and 2, and there was no serious adverse event related to the vaccine. At the same time, the

preliminary immunogenicity results of this study show that the positive conversion rate of live virus neutralizing antibody and the positive conversion rate of RBD binding antibody reached 100% in high and low dose groups after 7 days of injection.

The results of phase I clinical study showed that the safety and immunogenicity of the vaccine were good, and it could enter into the next large-scale clinical trial.

4.4.2. Recombinant Novel Coronavirus Vaccine (CHO cell) Phase II clinical trial

The Phase II clinical trial of the Recombinant Novel Coronavirus Vaccine (CHO cell) in healthy people aged 18 to 59 years old is a randomized, double-blind, placebo control study. The investigational vaccine groups include a low dose group (25 μg/ 0.5ml/vial) and a high dose group (50μg/ 0.5 ml/vial).

This trial includes healthy adults aged 18-59 with a total number of 900. There are 2-dose schedule (immunization at month 0, month 1) group (150 in the low-dose vaccine subgroup; 150 in the high-dose vaccine subgroup; 150 in the placebo subgroup) and 3-dose schedule (immunization at month 0, month 1 and month 2) group (150 in the low-dose vaccine subgroup; 150 in the high-dose vaccine subgroup; and 150 in the placebo subgroup). Blood samples of all subjects are collected before each dose, 14 days after each dose, 1 month, 6 months and 12 months after the full course of immunization for humoral immunogenicity analysis. Blood samples of the first 24 subjects in each group are collected before the first dose of inoculation, 4 days and 12 months after the full course of immunization for cellular immunogenicity analysis, and immunogenicity indexes of the two groups are compared. All subjects are also observed for safety from receiving the first dose of vaccine until 12 months after full course of immunization and are observed for immune persistence during 12 months after full immunization.

The database of Phase II trial was locked and analyzed on October 22, 2020. The scope of database lock-in included the visit data and partial immunogenicity data collected by EDC before October 21, 2020.

The preliminary immunogenicity results show that the positive conversion rate of live virus neutralizing antibody, GMT of live virus neutralizing antibody, positive conversion rate of RBD protein binding antibody and GMT of RBD protein binding antibody are all increased after injection, and the increasing trend became more

obvious with the increase of inoculation times.The levels of the above indicators on the 14th day after the third dose of investigational vaccine were significantly higher than those on the 14th day after the second dose of investigational vaccine, while the placebo group was consistent with that before immunization and maintained at a lower level, and the trend of the low and high dose group was roughly the same. On the 14th day after the third dose of vaccine, the above immunogenicity related indicators in the low-dose group were significantly higher than those in the high-dose group.

The preliminary safety results of this study show that: there were no deaths and serious adverse events related to the vaccine. The systemic adverse reactions were mainly fever and fatigue, and the local adverse reactions were mainly pruritus, erythema and swelling of the inoculation site. Most of them were mild and transient adverse reactions. No case of SUSAR occurred, which proved that the vaccine has good safety.

4.5. Rationale for the selection of vaccine dosage and immunization schedule basis for selection of dose and immunization schedule

The analysis of immunogenicity in the currently completed phase II clinical trial for this vaccine showed that the positive conversion rate of neutralizing antibody of live virus and GMT of neutralizing antibody of live virus were higher 14 days after vaccination of three doses at low dose as compared with two-dose groups (low dose, high dose and placebo), three-dose groups of high dose and placebo. It was indicated that low dose (25 μg /0.5 mL/vial) should be selected for the phase III clinical study on this product, given for three doses according to 0, 1 and 2-month immunization schedule.

The interim analysis of safety data in the currently completed phase I and II clinical trials for this vaccine showed that local and systemic adverse reactions occurred in the subjects participating in the trials, the systemic adverse reactions were mainly pyrexia, headache and asthenia and so on, local adverse reactions were mainly pain, swelling, induration and flushing, which were all slight and transient, and grade 1 and 2 in severity, no death or vaccine related serious adverse events occurred in the trials, showing a good safety profile for each dose and immunization schedule of this vaccine.

In summary of the above immunogenicity and safety findings, low dose (25 μg /0.5

mL/vial) was selected and three-dose immunization schedule on Month 0, 1 and 2 was selected as the vaccination regimen for the phase III clinical trial for this vaccine.

4.6. Benefits/potential risks of subjects

4.6.1. Known potential risks

Participation in this study may prevent the respiratory disease induced by novel Coronavirus infection (COVID-19), and similar with any other vaccine, its immune effect needs to be evaluated in clinical trials; meanwhile, a part of subjects will receive placebo in this study, i.e., they will not have the protection from COVID-19, thus they may suffer from it for natural infection of novel Coronavirus during the observation.

At the same time, in some circumstances, the antibody may play a role in enhancing virus infection during viral infection, assist virus in entry into target cells and improve infection rate, this phenomenon is known as antibody dependence enhancement (ADE). In the last century, admission for vaccine enhanced disease (VED) was found in 80% subjects in the clinical trial on respiratory syncytial virus vaccine, and two subjects died finally. As a higher pathological injury score in lungs was observed after vaccination as compared with placebo group in the preclinical animal study (rhesus monkeys) for SARS vaccine, suspected or confirmed COVID-19 cases need to be closely noted in this clinical study. Subjects must be complaince with the local prevention and control requirements and go to hospital for diagnosis and treatment if they are suspected or confirmed to be infected with SARS-COV-2 during the study.

The potential risks for vaccination of Investigational product are only limited to the common adverse reactions of any vaccine for injection, for example, mild pain at the injection site and occasionally mild to moderate flushing, swelling and induration. Fever and anorexia may also occur, however, they are expected to be mild. Generally, they will relieve and disappear spontaneously, without treatment; the reactions may severe (e.g., high fever, allergic reaction, etc.) in individual subjects, which will be closely observed by investigators and undergo symptomatic treatment.

Blood collection is needed during the study, pain or ecchymosis may occur after blood collection.

4.6.2. Known potential risks

Subjects may get potential protection for vaccination of Investigational product, i.e., prevention from the respiratory disease induced by novel Coronavirus infection (COVID-19). Subjects will make contributions to the early approval of COVID-19

vaccine and benefit wider population through participation in this registration study.

4.7. Relevant definitions

(1) "Month" in visit: defined as "30 days".

(2) Woman at childbearing age: defined as the woman who is in a specific period from maturity of female reproductive organs (menarche) to ovarian failure (menopause).

(3) 18-59 years old: 18 years old (i.e., the date of 18 years old) and less than 60 years old (i.e., one day before 60 years old) on the day of enrollment.

(4) 60 years old and above: 60 years old (i.e., the date of 60 years old) and above on the day of enrollment.

5. Study Objectives and Endpoints

5.1. Study objectives

5.1.1. Primary objective:

To evaluate the protective potency and safety of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in the population aged 18 years and above.

5.1.2. Secondary objectives:

(1) To evaluate the protective potency of recombinant COVID-19 vaccine (CHO cell) in prevention of severe and critical COVID-19 in the population aged 18 years and above.

(2) To evaluate the immunogenicity and immune persistence of recombinant COVID-19 vaccine (CHO cell) in the population aged 18 years and above.

(3) To evaluate the protective potency of emergency vaccination of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in the population aged 18 years and above.

(4) To evaluate the protective potency of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in different age groups (18-59 years old, 60 years old and above).

5.1.3. Exploratory objectives:

To explore the immunological surrogate variables of recombinant COVID-19 vaccine (CHO cell) in prevention of COVID-19 in the population aged 18 years and above.

5.2. Study endpoints

5.2.1. Primary study endpoints:

(1) Study endpoint of protective potency:

Number of patients with any grade of COVID-19 14 days after full course of vaccination.

(2) Safety endpoints:

1) Analysis of adverse events from the first dose of vaccination to 30 days after full course of vaccination: incidence of adverse reactions and adverse events; incidence of grade 3 and above adverse eventsand above adverse reactions; incidence of adverse events or adverse reactions leading to withdrawal.Analysis of serious adverse events from the first dose of vaccination to 12 months after full course of vaccination: incidence of serious adverse events; incidence of serious adverse events related with the Investigational product

5.2.2. Secondary study endpoints:

(1) Study endpoint of protective potency:

1) Number of patients with severe and critical COVID-19 14 days after full course of vaccination;
2) Number of patients with any grade of COVID-19 14 days after the first dose of vaccination;
3) Number of patients with any grade of COVID-19 14 days after full course of vaccination in different age groups (18-59 years old, 60 years old and above).

(2) Study endpoints of immunogenicity and immune persistence:

SARS-COV-2 neutralizing antibody, RBD protein binding antibody IgG level on Day 14 , 6 months after full course of vaccination.

5.2.3. Exploratory endpoints:

SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG protective level against COVID-19 induced by novel Coronavirus infection.

6. Study Design

6.1. Overall design

A randomized, double-blind, placebo-controlled international multicenter clinical trial design will be adopted. A total of 29000 subjects aged 18 and above will be recruited, including 750 subjects aged 18-59 and 250 subjects aged 60 and above in China;

21000 subjects aged 18-59 and 7000 subjects aged 60 and above will be recruited outside China. Safety and immunogenicity will be evaluated in Chinese subjects, and protective efficacy, immunogenicity and safety will be evaluated in subjects outside China. Among them, 750 subjects aged 18-59, 250 subjects aged 60 and above and all subjects in China will be selected as immunogenicity subgroup. There will be 1000 cases in total, 500 cases in each group will be vaccinated with the investigational vaccine or placebo (the subjects in the immunogenicity subgroup participated in the evaluation of protection efficacy); meanwhile, an immunogenicity research cohort will be set up in China, with 500 cases in each group being vaccinated with the investigational vaccine or placebo. The levels of neutralizing antibody of SARS-COV-2 and IgG of RBD protein binding antibody will be detected before immunization, 14 days after the whole immunization, and 6 months after the whole immunization. The study will follow the requirements in ICH-GCP, subjects need to be informed of the background, content, potential risks and benefits of the study prior to the start of the study. Subjects need to provide written informed consent form, investigators should use local language to explain the risks and benefits of the study to subjects, including potential side effects of the vaccine. Subjects will also be told to provide corresponding biological samples for testing. Subjects also need to provide their own clinical data to investigators, however, these data will be confidentially saved and processed in accordance with local national and international criteria and regulatory requirements.

Screening eligible subjects will obtain one study number and be randomized to placebo control group or Investigational product group. The vaccination will be performed in accordance with Month 0, 1,2 vaccination procedure at the dose of 25μg/0.5mL/vial , and the follow-up visit will continue for 14 months.

6.2. Criteria on study suspension or termination

Acquisition of new data on the Investigational product from this study or any other study that is considered by regulatory authorities, the sponsor, investigators and/or institutional review board /Independent Ethics Committee as requiring suspension/termination of the study;

Criteria on study suspension:

In case of the following conditions, the study needs to be suspended, and the institutional review board/independent ethics committee, relevant regulatory authorities will be reported immediately, data safety monitoring board (DSMB) expert

meeting will be held urgently for safety demonstration, analysis and determination on whether to continue this study.

Adverse event leading to study suspension	Number/%
≥grade 3 adverse reaction for 48 hours, following any dose-time of vaccination	>20% vaccinated persons

Criteria on study termination:

In case of the following conditions, the study needs to be terminated, and the institutional review board/independent ethics committee, relevant regulatory authorities will be reported immediately.

Adverse event leading to study termination	Number/%
≥grade 3 adverse reaction for 48 hours, following any dose-time of vaccination	>30% vaccinated persons

If the study is suspended or terminated prematurely, the sponsor will inform investigators, the ethics committee and drug regulatory authorities of the reason for the suspension or termination immediately in accordance with the requirements in corresponding registration regulations.

Regardless of the reason for early termination of the study, investigators should inform subjects immediately, and ensure appropriate follow-up of subjects.

6.3. ADE/VED (antibody dependence enhancement/ vaccine enhanced disease) risk monitoring

Following vaccination (at least one dose of Investigational product). If the subject is diagnosed as COVID-19, he/she should be hospitalized or isolated in accordance with requirements for epidemic prevention and control in that region. Special investigation is needed for severe or dead cases, and DSMB will analyze the presence of ADE/VED according to the results of investigation.

7. Monitoring of COVID-19 case

7.1. Diagnostic criteria and definition

(1) COVID-19 diagnostic criteria: SARS-COV-2 infection confirmed in laboratory (SARS-COV-2 nucleic acid positive as detected using real-time fluorescence quantitative RT-PCR).

(2) COVID-19 of Any Severity: COVID-19 infection confirmed in laboratory, and having at least one of the following symptoms: fever (axillary temperature ≥37.3℃, oral temperature ≥37.5℃), cough, shortness of breath, chills, fatigue,

myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell / taste.

(3) Suspected COVID-19: Those who meet any of the following conditions: (1) having fever (axillary temperature ≥37.3°C,oral temperature ≥37.5°C), cough and shortness of breath; (2) having chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell / taste for consecutive two days or above; (3) having the symptoms described in (2) but not lasting for two days or above, judged by investigators as suspected case based on their own clinical experience, inquiry of history of present illness or epidemiological history.

(4) Mild COVID-19: COVID-19 infection confirmed in laboratory, being symptomatic without evidence on viral pneumonia or hypoxia. The symptoms include fever, cough, fatigue, anorexia, shortness of breath, myalgia, sore throat, stuffiness, headache, diarrhea, nausea, vomiting, loss of smell (anosmia), loss of taste (ageus).

(5) Common COVID-19: COVID-19 infection confirmed in laboratory, having clinical signs of pneumonia (fever, cough, dyspnea, shortness of breath) but no signs of serious pneumonia, including SPO2≥ 90% under indoor conditions.

(6) Severe COVID-19: COVID-19 infection confirmed in laboratory, and meet any of the following conditions:1) In case of shortness of breath, RR ⩾ 30 times / min; 2) under resting state, oxygen saturation ⩽ 93% during air inhalation; 3) arterial partial pressure of oxygen (PaO2) / oxygen inhalation concentration (FiO2) ⩽ 300 mmHg (1mmhg = 0.133kpa); PaO2 / FiO2 should be corrected according to the following formula: PaO2 / FiO2 X [760 / atmospheric pressure (mmHg)]; 4) the clinical symptoms worsened progressively, and the lung imaging showed that the lesions progressed more than 50% within 24-48 hours.

(7) Critical COVID-19: COVID-19 infection confirmed in laboratory, acute respiratory distress syndrome, sepsis, septic shock, other complications in the patients with COVID-19, including acute and life-threatening conditions, e.g., acute pulmonary embolism, acute coronary syndrome, acute stroke and delirium.

7.2. Case monitoring, diagnosis and treatment

7.2.1. Diagnostic procedure for suspected cases

Symptom-driven passive monitoring is used for monitoring of COVID-19 cases. Prior

to the start of the study, investigators should establish all kinds of feasible communication channels, including network, telephone, text message, face-to-face interview, make sure the subjects can communicate with investigators at any time, be familiar with the monitoring and diagnostic procedure of COVID-19 cases, and implement personal protection strictly. Train investigators on all methods of detection of Novel Coronavirus nucleic acid to ensure the authenticity and reliability of results and reduce differences. Following the first dose of vaccination, the following procedure will be initiated if the subject has the symptoms consistent with suspected COVID-19.

1. Subjects will contact investigators immediately (face-to-face interview or telephone, text message, network, etc.).
2. Investigators will confirm if the definition of suspected case is met based on the symptoms provided by subjects and fill in case report forms.
   (1) If yes, subjects will arrive at the study institutions or designated medical institutions as early as possible for the following examinations, as instructed by investigators. The biological sample (oropharyngeal swab) will be collected by study personnel on site for real-time fluorescence quantitative RT-PCR;
      a. If the test result is positive, the subject will be one COVID-19 case and managed in accordance with local epidemic diagnosis and treatment measures.
      b. If the test result is negative, the subject will be followed up by investigators; if the symptoms are still ongoing or the original symptoms are exacerbated or new relevant symptoms appear within two days, the sample will be re-collected (at least 48 hours interval with the previous nucleic acid detection time) for fluorescence quantitative RT-PCR. If it is positive, the subject will be one COVID-19 case and managed in accordance with local epidemic diagnosis and treatment measures; if it is negative, the subject will be one non-COVID-19 case, the study personnel will provide advice, for example, routine medical treatment.
      c. If the test result is negative, and no above symptoms occur within two days, the subject will be one non-COVID-19 case, the study personnel will provide advice, for example, routine medical treatment.
   (2) Otherwise investigators will provide advice, for example, routine medical

treatment.

7.2.2. Judgment for severity of COVID-19

For the subjects who are diagnosed as COVID-19, they should be treated and cured at the study institutions or designated medical institutions as far as possible according to local therapeutic regimen if hospitalized, investigators should collect the clinical data of relevant examinations and diagnoses during hospitalization (see Appendix C); if not hospitalized, subjects shall be treated according to the local epidemic diagnosis and treatment measures, and home care should be taken to take personal and family epidemic prevention measures,followed up by investigators through face-to-face interview, telephone, text message or network, as to collect the data on the course of disease, until its outcome. The severity will be judged based on the data on the course of disease or the data consistent with the criteria on critical illness (note: subjects without critical illness need to be followed up until its outcome, the severity will be judged in accordance with the most serious point in the course of disease in combination with the definition of severity of COVID-19, if the subject has critical illness, i.e., reaching the judgment point, the severity can be judged with no need to wait for its outcome).

Start time of disease: the occurrence time of the first symptom or sign at the first diagnosis of COVID-19 in laboratory. For example, one subject reports cough and fever to investigators on August 1, COVID-19 is diagnosed in the laboratory on August 3, the start time of disease should be recorded as August 1 for this subject.

End time of disease: the time when the disease is cured (based on the criteria on cure in each country); in case of death, the time of death will be used as the end time; if the subject still can not be cured at the end of the study, the end time will be left blank and the outcome will be recorded as “still ongoing”.

7.2.3. Review of COVID-19 case

All the clinical data and case report form from the cases diagnosed as COVID-19 will be submitted to EAC in accordance with the time points and procedure specified in Endpoint Adjudication Committee (EAC) constitution, EAC will check COVID-19 cases and the severity of COVID-19, the final results will be based on the evaluation by EAC.

7.3. Time of case monitoring

All the subjects receiving the vaccination will be monitored for COVID-19 during the study. The data on COVID-19 monitored from the 1st dose of vaccination to one year

after the full course of vaccination will be statistically analyzed, and used for the registration application of the vaccine.

7.4. Collection of biological samples

1) Oropharyngeal swab biological sample will be collected from the subjects with suspected COVID-19 reported at each study site (based on the SOP for collection of biological samples).
2) The biological sample collected at each site will be divided in duplicate, one for real-time fluorescence quantitative RT-PCR at the laboratory for detection of novel Coronavirus nucleic acid (based on the SOP for detection of novel Coronavirus nucleic acid); the other one archived and stored in qualified laboratory for back-up.

8. Study Population

8.1. Study subjects

Study subjects are ≥18 years old.

8.2. Inclusion criteria

(1) Subjects ≥18 years old;
(2) Subjects who voluntarily participate in the study and sign the informed consent form, and can provide the effective identity certificate, understand and follow the requirements of the study protocol;
(3) Female subjects of childbearing age who agree to use the effective contraceptive measures from the starting of the study to 6 months after the completed inoculation.

8.3. Exclusion criteria

(12) Suspected or confirmed as fever within 72 hours before the enrollment, or axillary temperature ≥37.3°C / oral temperature ≥37.5°C at the day of screening;
(13) Diastolic blood pressure ⩾ 100mmhg and / or systolic blood pressure ⩾ 150mmhg;
(14) Patients with previous history of a COVID-19;
(15) Detection of SARS-COV-2 nucleic acid or antibody is positive;
(16) Those who are suffering from the following diseases:

a) With thrombocytopenia, any coagulation dysfunction or receiving anti-coagulatory treatment

b)Congenital or acquired immune deficiency or autoimmune disease history;

no spleen, or history of splenic surgery and trauma, or receiving immunomodulator treatment within 6 months, e.g., immunosuppressive dose of glucocorticoids (reference dose: equivalent to 20mg/ day of prednisone, over 1 week); Or monoclonal antibodies; Or thymosin; Or interferon etc.; However, topical application (such as ointment, eye drops, inhalers or nasal sprays) is permitted;

c)Symptoms related to acute respiratory tract infection (such as sneezing, nasal congestion, runny nose, cough, sore throat, loss of taste, chills, shortness of breath, etc.);

d)Cancer patients (except basal cell carcinoma)

(17) With a history of serious allergy to any vaccine or any composition of Investigational product (including: aluminum preparations), such as allergic shock, allergic throat edema, allergic purpura, thrombocytopenic purpura, localized allergic necrosis reaction (Arthus reaction), dyspnea and angioneuroedema;

(18) Inoculated with subunit vaccine and inactivated vaccine within 14 days before the first dosing of investigational? vaccine, or inoculated with attenuated live vaccine within 30 days;

(19) Previous receiving blood transfusion or blood relevant products (including immunoglobulin) within 3 months, or planning to receive such products from the starting of study to <6 months after the whole-course inoculation;

(20) Have participated in or are participating in other covid-19 related clinical trials;

(21) Women in breastfeeding period or in pregnant period (including women at childbearing age with positive result of urine pregnancy test);

(22) Considered by investigators as any disease or state possibly making the subject at unacceptable risk; not conforming to the requirements of study protocol; interference of assessment of reactions of vaccine.

9. Study Procedures

Subjects must read and sign an informed consent form approved by the Ethics Committee prior to the study. Each examination and study procedure are performed in accordance with the study flowchart.

9.1. Informed consent

The subjects must sign the latest approved version of informed consent from with

noting a date before any study procedure is performed.

The contents of ICF include at least: overview of clinical trial; objective of trial; tentative contents involved in clinical trial; investigational treatment and possibility for random allocation into each group; procedures of trial to be observed by subjects; predicted benefit of trial; and possibility for unable benefit., risks of subjects; expenses to be paid; compensation; scope of use and confidentiality of samples

The subject can withdraw from study for any reason and at the same time, their legitimate rights and interests shall not be affected in any way.

The subject will possess sufficient time to consider such information and have an opportunity to inquire investigators or other independent party for deciding on whether to participate in this study. The investigators must possess the relevant qualification and experience, and obtain the authorization of PI. Then, ICF is jointly signed by investigators and subjects, which is in duplicate. A copy of the signed ICF will be given to the subject. The signed original is kept in study site.

9.2. Evaluation of inclusion and exclusion criteria

Visit 1 (D-7~0): After ICF of latest approved version is signed by subjects, screening number is assigned by investigators; demographic data are collected (sex, birth date, height and weight); medical history (past illness and present illness), allergic history and concomitant medicationsconcomitant medication are inquired, physical examination (skin and mucous membranes, lymph nodes, head, neck, chest, abdomen, spine / limbs)axillary temperature is measured; orophsryngeal swab is collected for nucleic acid test on SARS-COV-2 (real-time fluorescence quantitative PCR, RT-PCR); blood sample is collected for antibody test on SARS-COV-2 (colloidal gold technique); inclusion/exclusion criteria are verified.

Visit 2 (D0): Medical history (past illness and present illness), allergic history and concomitant medicationconcomitant medication, and perform vital signs assessment (blood pressure, axillary / oral temperature, pulse) are inquired by investigators again; axillary temperature is measured; in female subjects at childbearing age, urine pregnancy test is made. According to the inclusion/exclusion criteria, the subjects are assessed by investigators; and the subjects conforming to inclusion criteria are determined.

9.3. Randomization and blinding

9.3.1. Randomization

Stratified block randomization is used in this trial, subjects are stratified by center

(participating country) and age (18~59 years, 60 years and above), randomized allocation of subjects and Investigational product are completed using Interactive Web Response System (IWRS).

SAS 9.4 or above version software is used by randomization statisticians to generate subject randomization table and vaccine randomization table, which are imported into IWRS system by system engineer. Upon successful screening of subjects, the study personnel participating in this trial at each site will login IWRS system to acquire subject's random number; investigators will login IWRS system to acquire vaccine number prior to vaccination, and immunize according to vaccine number; in case of vaccine damage, investigators can acquire a new vaccine number from IWRS system and perform vaccination with the new vaccine number.

9.3.2. Blinding

Prior to the start of the study, the staff from the sponsor who are not involved in this clinical study will blind the Investigational product uniformly together with non-blind randomization statisticians, i.e., paste the printed label at the designated position of each vaccine according to the content of vaccine blindness. The blinding of vaccine will be supervised by randomization statisticians, who will guide blinding operators to label according to the content of blindness. Upon completion of blinding, the content of blindness should be sealed by non-blind randomization statisticians. The whole blinding process will be recorded and written in document form, i.e., blinding record, and kept as one of the important documents for this clinical study. The blinding personnel must not be involved in other relevant work in this clinical study or reveal the content of blindness to anyone who is involved in this clinical study.

This study includes one subject randomization table and one vaccine randomization table, the content of blindness including the parameter encoding corresponding group and the number of seed generating random number is sealed in duplicate and handed over to the sponsor and the unit in charge of the clinical study for preservation respectively, and should be kept properly until lock of database.

9.3.3. Emergency unblinding

In case of emergency, when the investigator considers knowing the vaccine used by the subject is conducive to the management of adverse events, the detailed group of the subject can be obtained through the emergency unblinding module in IWRS system. In case emergency unblinding is needed, relevant personnel from the sponsor must be notified in advance as far as possible prior to the unblinding of the investigational vaccine, if possible. In case it fails to contact the sponsor prior to emergency unblinding, the sponsor must be contacted within 24 hours after emergency unblinding. Corresponding cases will be processed as drop-out once emergency unblinding is performed. Investigators need to record the date and reason for unblinding, and the unblinding process in the source document.

9.3.4. Unblinding requirements

When all the data are entered and signed, and the analysis population is determined in the blind data review meeting, the database will be locked. Unblinding can be carried out only upon lock of database, the vaccine corresponding to the study number will be unblinded. The unblinding document will be signed jointly by the principal investigator, sponsor and statisticians.

9.4. Vaccination

Visit 2 (D0): After the subjects passing the screening are grouped randomly, the vaccine or placebo is injected intramuscularly into deltoid muscle at upper arm. In order to ensure the safety of subjects, the subjects are retained for observation of 30 minutes after the inoculation; and all adverse events occurring during this period are recorded by investigators.

Before the subjects leave study field, the subjects are trained by investigators on the completion of diary card; straight ruler and thermometer is granted; the subjects are guided to measure axillary temperature and oral temperature through the straight ruler and thermometer; and the reactions at the site of injection are recorded. After the subjects leave study field, the axillary temperature, concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 0~7 days after the inoculation are recorded according to relevant requirements; At the same time, notified cards will be issued to the subjects, including the symptoms related to the suspected cases and the contact information of the investigators. The investigators will explain to the subjects. If the symptoms in the reminder card appear after the subjects leave the site, they need to contact the investigators in time, and the investigators will give advice on whether they need to go to the institute or the designated institution for COVID-19 nucleic acid detection, if the subject left the test site, otherabnormal conditions need be contacted in time after the subjects leave study field, the adverse events occurring after this inoculation and the suspected cases of COVID-19 are treated by investigators in time; original medical record is completed.

At Day 0 of study, in the immunogenicity subgroup of subjects passing the screening, 5 mL of blood sample is additionally collected before the inoculation for immunogenicity test.

9.5. Follow-up

Concrete information on follow-up is shown in Attachment A: Study procedures for

subjects.

Visit 3 (V2+8): The diary card is collected by investigators; the contents of diary card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Contact card is granted by investigators to subjects; the subjects are trained on the completion of contact card; the contact card is used to record the concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 8~30 days after the inoculation.

Visit 4 (V2+30): The contact card is collected by investigators; the contents of contact card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Axillary temperature/oral temperature of subjects is measured. After the assessment and confirmation of investigators, the vaccine or placebo is inoculated for the second dosing into subjects. In order to ensure the safety of subjects, the subjects are retained for observation of 30 minutes after the inoculation; and all adverse events occurring during this period are recorded by investigators. Before the subjects leave study field, the diary card is granted; the axillary temperature and /oral temperature, concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 0~7 days after the inoculation are recorded.

Visit 5 (V4+8): The diary card is collected by investigators; the contents of diary card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Contact card is granted by investigators to subjects; the contact card is used to record the concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 8~30 days after the inoculation.

Visit 6 (V4+30): The investigator collected the contact cards, reviewed the contents of the contact cards and signed for confirmation, evaluated the adverse events and filled in the original medical records. The axillary temperature / oral temperature of the subjects will be detected, and the subjects will be given the third dose of vaccine or placebo after the evaluation and confirmation of the investigators. To ensure the safety of the subjects, the subjects will be observed for 30 minutes after injection, during which all adverse events occurred within 30 minutes after vaccination were recorded by the investigators. A diary card will be issued before the subjects left the site to record the axillary / oral temperature, concomitant medication and adverse events (both the solicited and the unsolicited adverse events) at 0-7 days after

injection.

Visit 7 (V6 + 8) - the investigator collected the diary card, reviewed the contents of the diary card and signed for confirmation, evaluated the adverse events and filled in the original medical record. A contact card was issued to the subjects to record the drug combination and adverse events (non solicitation adverse events) 8-30 days after vaccination.

Visit 8 (V6+14): In the immunogenicity subgroup of subjects, 5 mL of blood sample is collected for immunogenicity test.

Visit 9 (V6+30): The contact card is collected by investigators; the contents of contact card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed.

Visit 10 (V6+180): In the immunogenicity subgroup of subjects, 5 mL of blood sample is collected for immunogenicity test.

Visit 11 (V6+360): Telephone visit, serious adverse events occurring from the inoculation for first dosing to <12 months after the whole-course inoculation are collected by investigators.

9.6. Concomitant medications

At each visit/contact, the subjects are inquired by investigators on whether any drug has been given and any vaccine has been inoculated. All concomitant medications/vaccines are recorded (except vitamin and/or food additives). The concomitant medications are transcribed by investigators into eCRF.

Combined drugs mean all drugs (except Investigational product) given into subjects from the signing of ICF to <30 days after the inoculation for last dosing and all drugs (except Investigational product) given due to the SAE and pregnancy from 30 days to 1 year after the inoculation for last dosing, including: antibiotics, antiviral drugs, antipyretic analgesics, anti-allergic drugs, biological products (vaccine), and Chinese (patent) medicines (except vitamin and/or food additives).

The concomitant medications are classified by data administrator into the following 10 categories:

(1) Hormones/steroids and other immunosuppressants;
(2) Antiallergic drugs;
(3) Antipyretics/analgesics/non-steroidal anti-inflammatory drugs;
(4) Vaccines and biological products;
(5) Immune globulins and other blood products;

(6) Antibiotics;

(7) Antivirals;

(8) Chinese patent medicine;

(9) Recipe of Chinese medicine

(10) Others.

Allowable vaccine: The vaccine is used by observing the inclusion/exclusion criteria; for the emergency inoculation of vaccine (such as rabies or tetanus), such limitation should not be required, but the use conditions of vaccine should be recorded strictly by the facts according to the relevant requirements. Inoculation of other vaccines before the inoculation of Investigational product: The subunit vaccine and inactivated vaccine is inoculated by interval of at least 14 days from the inoculation of Investigational product; the attenuated live vaccine is inoculated by interval of at least 30 days from the inoculation of Investigational product.

Allowable medications: During the trial, necessary medications shall be allowed for treatment if the subject develops any adverse events, and the medication information shall be recorded according to the requirements. If contraception requirements are raised for subjects during study period, contraceptives can also be allowed; but the information on any used drug should be recorded strictly by the facts according to the relevant requirements.

Preventive drugs: mean the drug given when there are no symptoms or anticipated occurrence of vaccination reactions. If Aspirin is used by subjects for treating heart disease, the used Aspirin is a type of concomitant medications to be reported, but is not a type of preventive drugs. If antipyretics is given for fever prevention in the subjects without fever during the recruitment period, the antipyretics is considered as a type of preventive drugs. At enrollment, the subjects are inquired on the ongoing drugs to confirm that antipyretics, analgesics or anti-allergic drugs are not given.

9.7. Biological samples processing

For immunogenicity test, about 5 mL of blood sample is collected by avoiding a hemolysis. The centrifuged serum is subpacked into 4 freezing tubes (about 250 μL each tube), including: 3 tubes sent for examination and 1 tube for reservation; after the marking, these tubes are kept at -20°C (treatment procedures for biological sample are shown in the treatment SOP for biological samples).

9.8. Detection of biological samples

The inspection quality control standard is provided by the National Institutes for Food

and Drug Control. The test on SARS-COV-2 neutralizing antibody and RBD protein bindin antibody is completed by National Institutes for Food and Drug Control of China.

9.9. Withdrawal criteria

Subjects may withdraw from the study at any time. Besides, the investigator can require the subject to withdraw from the study at any time for following reasons:

- Occurrence of AEs or complicated conditions unable to continue the study;
- Participating in other clinical trials before the termination of this clinical trial;
- Subject s with other conditions that are not suitable to participate in the clinical trial, as considered by the investigator.

The reasons for study withdrawal are recorded into case report form (CRF); the withdrawn or rejected subjects will not be replaced.

9.10. End of study

The ending date of study in 1 year after the whole-course inoculation of vaccine in last subject.

10. Investigational product

10.1. Description of Investigational product

10.1.1. Recombinant Novel Coronavirus Vaccine (CHO Cell)

The Recombinant Novel Coronavirus Vaccine (CHO cell) prepared by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd ("Zhifei Longcom") is produced by recombinant CHO cell expressing the receptor binding region of the CoronavirusNovel Coronavirus spike glycoprotein (recombinant protein NCP-RBD) after purification, with aluminum hydroxide adjuvant added. The product can be stratified by precipitation and after shaking, it is easy to disperse. "Novel Coronavirus Vaccine (CHO Cell) Manufacturing and Verification Regulations (Draft)" has passed the verification of both the company and China food and Drug Control Institute. The relevant information and content are as follows:

Name:	Recombinant Novel Coronavirus Vaccine (CHO cell)
Manufacturer:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd
Batch number:	See Quality Inspection Report

Specifications:	0.5ml/vial. It contains 50 μg NCP-RBD protein
Ingredients:	NCP-RBD Aluminum Hydroxide Adjuvant
Other:	See Quality Inspection Report
Inspection Institution:	National Institutes for Food and Drug Control
Inspection Report No.:	See quality inspection report
Shelf Life:	2 years(provisional)

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible institution shall explain and report to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.1.2. Novel Coronavirus Vaccine Placebo

The placebo, produced by Anhui Zhifei Longcom Biological Pharmaceutical Co., Ltd., does not contain any effective ingredients of the COVID-19 vaccine. The ingredients and contents are as follows:

Name:	Novel Coronavirus Vaccine (CHO cell) placebo (with aluminum)
Manufacturer:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd
Batch number:	See Quality Inspection Report
Specifications:	0.5 ml/vial. It contains 0.25mg aluminum hydroxide adjuvant
Ingredients:	Aluminum hydroxide adjuvant
Other:	See Quality Inspection Report
Inspection Institution:	National Institutes for Food and Drug Control
Inspection Report	See Quality Inspection Report

No.:	
Shelf Life:	2 years(provisional)

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible institution shall explain and report to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.2. Management of Investigational product

The institution in charge of vaccine clinical trial needs to guide the study site to formulate the management system of Investigational product, the management of receipt, safekeeping, recovery, return/destruction of Investigational product needs to meet the requirements in relevant SOP. The institution in charge of vaccine clinical trial and study site needs to designate the personnel trained on GCP and relevant knowledge to take charge of management of Investigational product.

Vaccine transportation: the full course of vaccine management needs to meet cold-chain requirements, vaccine transportation and storage conditions as required in the protocol must be available. The vaccine will be stored and transported at 2-8℃, protected from light, a delivery note and temperature monitoring must be available during vaccine transportation, the packaging and unpacking temperature will be recorded on arrival, the receiver will sign on the delivery note and fax or copy to the shipper upon verification of the vaccine, the delivery note will be kept properly by both parties.

Vaccine storage and distribution: the Investigational product will be stored in separate partition at 2-8℃, protected from light, managed according to the locking requirement by specially-assigned person in special counter, blind management needs to be maintained for blind study. The vaccine receiver must check the vaccine delivery status, establish worksheets of vaccine handover, registration, use and recovery, fill in it as required and keep in the work record.

Record of vaccine handover: the Investigational product will be provided by the sponsor, investigators will check the name, dosage and package of the vaccine whilst receiving them, and make a record of handover.

Record of vaccine registration and use: the record of vaccine registration and use will be formulated by investigators. The vaccines distributed to each subject should be well recorded, including the study number, subject initials and signature of vaccinator.

Record of vaccine retrieving: the vaccine administrator needs to recover the residual vaccines in time, place them separately, count and complete the inventory record on a regular basis, and the inconsistencies between the used vaccines and residual amount and the total number should be explained. The discarded, expired and residual vaccines in this study will be returned to the sponsor, the external packing of all the

used vaccines needs to be retained for inspection during the study. The sponsor will verify the vaccine amount whilst receiving them and make relevant records well, which will be signed by vaccine administrator and the sponsor's representative. The sponsor must keep the vaccine at least until completion of NMPA verification according to cold-chain requirements.

Cold-chain failure: once the temperature for vaccine storage is <2℃ or >8℃, it will be regarded as cold chain failure. The sponsor needs to elucidate the information on vaccine stability in the investigator's brochure, once cold chain failure occurs, investigators should transport the vaccine to a dark environment at 2~8℃ for storage, use of the vaccines suffered from cold-chain failure should be suspended, and the sponsor should be reported as early as possible, discontinuation or continuation of the use of vaccine will be determined based on the written opinion from the sponsor. The vaccines suffered from cold-chain failure can not be used for subjects prior to acquisition of the sponsor's opinion on their disposal.

The investigational vaccine and control vaccine can not be used for non-clinical trial population.

11. Safety Report

11.1. Definitions

Adverse Events (AE)	Referring to any accidental medical accident occurred in the subject after he/she receiving the Investigational product treatment, including those accidents not necessarily caused by or related to the product.
Solicited Adverse Events	Adverse events collected as safety endpoints in the study, referring to the adverse events information collected by investigators or subjects during a specific follow-up period after injection.
Unsolicited Adverse Events	Other adverse events reported in the study other than solicited adverse events, also include those reported not during the designated solicited adverse reaction time window.
Adverse Reactions (AR)	Referring to any harmful or unexpected reaction that may be related to the Investigational product in clinical trial. There is at least one reasonable possibility, which can not be excluded, of the causal relationship between the Investigational product and the adverse event.

Serious Adverse Events (SAE)	Referring to the adverse medical events such as death or life-threatening conditions, permanent or serious disability or function loss, the need for hospitalization or extended hospital stay, and congenital abnormalities or birth defects after receiving the Investigational product.
Serious Adverse Reactions (SAR)	Referring to an adverse event that is both serious and considered to be an vaccine adverse reaction.
Suspected Unexpected Serious Adverse Reactions (SUSAR)	Referring to the suspicious and unexpected serious adverse reactions with the nature and severity that is exceeding the existing available information such as the investigator's brochure of the investigational vaccine, instruction of marketed drug or the synopsis of the product characteristics.
Anticipation	The investigator and the sponsor should determine whether the serious adverse events associated with the trial vaccine are expected or unexpected. If the nature, severity or frequency of the adverse event is inconsistent with the risk information of the previously described study intervention, it should be considered unexpected.

11.2. Correlation with Investigational product

For the expected or unexpected AE (solicited or unsolicited AE), the investigator should take measures to judge the correlation with vaccination in time, timely discover SAE and mass and tendentious adverse events related to vaccination in the clinical trial, and timely suspend or terminate the clinical trial in order to minimize the harm to the subjects.

Definitely Relevant	There is clear evidence of causality, and other possible contributing factors can be excluded.
Highly Likely Relevant	There is evidence of a causality, and other factors are unlikely to be involved.
Possibly Relevant	There is some evidence of causality (e.g., the event occurs within a reasonable time after administration of the Investigational product). However, the influence of other factors may lead to the

	occurrence of the event (such as the clinical conditions of the patients, other concomitant treatment).
Possibly Not Relevant	There is little evidence of a causality relationship (e.g., the event did not occur within a reasonable time after administration of the Investigational product), or there is another reasonable explanation for the event (such as the clinical conditions of the subjects, other concomitant treatment).
Definitely Irrelevant	There is no evidence of any causality

11.3. Solicited adverse events

Solicited AE: The following events occurred within 7 days after injection:

Injection site (local) adverse events	Pain, swelling, induration, redness, rash, pruritus
Vital signs	fever
Non injection site (systemic) adverse events	Headache, fatigue / fatigue, nausea, vomiting, diarrhea, muscle pain, cough, acute allergic reaction, mental disorder (specific symptoms)

11.4. Unsolicited adverse events

Unsolicited adverse events are all adverse events, other than solicited adverse events, which reported during the period from the first dose of vaccination to 30 days after the whole vaccination, and also include the adverse events reported outside the designated recruitment time window (for example, if the above-mentioned solicited adverse events occur on or after the 8th day of vaccination, it will be recorded as an unsolicited adverse event)

11.5. Recording procedure for adverse events

The AE grading standard of this study will be recorded and evaluated on the basis of the Guideline on the Classification of Adverse events in Clinical Trials of Preventive Vaccine issued by the National Medical Products Administration (NMPA) and in combination with the requirements of regions outside China .

During the trial, adverse events observed by investigators or reported by subjects will be recorded on the case report form (CRF), whether or not related to the investigational vaccine.

The following information will be recorded: the name of the adverse event, the date of onset and end, the severity, the evaluation of correlation between the AE and the vaccine, the concomitant medication and non-medication treatment, and the measures taken. Follow up information should be provided if necessary.

The adverse events assessed by qualified medical investigators as relevant to the investigational vaccine should be followed up until the events end or stabilize.

It is the investigator's clinical assessment to determine whether the severity of AE requires the subject to withdraw from trial. Subjects may also voluntarily withdraw from treatment due to adverse events that they consider intolerable. In case of any of the above conditions, the subjects must be assessed at the end of the trial and given appropriate care under medical supervision until the symptoms stop or the conditions stabilize.

11.6. Reporting of serious adverse events

11.6.1 Time of reporting

All the procedures for SAE reporting will be carried out by on-site PI in accordance with local/national ethics committee and regulatory requirements, however, the SAE should be reported to the sponsor within 24 hours. Upon receipt of safety related information, the sponsor should analyze and evaluate it immediately, including the seriousness, correlation with the investigational product and whether it is one expected event.

11.6.2 Content of report

1) Type and time of report (initial report, follow-up report, summary report and corresponding time of report);
2) Subject’s information (name initials, study number, date of birth, gender);
3) Reporter’s information (name of medical institution and specialty, telephone, position / title);
4) Information on the suspected drug (Chinese and English names, registration classification and dosage form);
5) Study related information (clinical study approval letter number, clinical study classification, clinical trial indication);
6) Concurrent disease and therapy information (name of diagnosis, name and administration and dosage of therapeutic agent);

7) Detailed information on SAE (name of diagnosis, whether it belongs to ADE/VED, seriousness criteria, time of onset, end time, laboratory examination findings, course of therapy, prognosis, measures taken for the investigational vaccine and correlation with the investigational vaccine);

8) Unblinding;

9) Time of awareness by the investigator;

10) Signature of the investigator.

11.7. Suspected unexpected serious adverse reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) needs to meet the following three criteria at the same time:

1) Suspected adverse reaction: defined as the harmful reaction unrelated with the administration at any dose, its correlation with the drug is considered to be at least possibly related through analysis.

2) Unexpected adverse reaction: defined as an adverse reaction whose nature, severity, consequence or frequency is not consistent with the description of the anticipated risk in the previous protocol or other relevant materials (e.g., investigator's brochure). As the master document, the investigator's brochure provides the reference safety information to judge if one adverse event is expected or unexpected.

3) Serious adverse reaction: defined as an adverse reaction whose seriousness reaches the criteria on serious adverse reaction, including one of the following conditions: death, being life-threatening, permanent or serious disability or loss of function, requiring hospitalization or prolonged hospital stay, as well as congenital anomaly or birth defects after administration of Investigational product.

The sponsor will submit the initial report to the Center of Drug Evaluation of China Food and Drug Administration, provincial food and drug administration and inform the principal investigator at all the clinical trial institutions in the following time limits based on the nature (category) of SUSAR event.

1) For fatal or life-threatening suspected unexpected serious adverse reaction (SUSAR), the sponsor should report it after awareness of it for the first time as soon as possible and no later than 7 natural days, and report relevant follow-up information within the following 8 natural days.

2) For non-fatal or life-threatening suspected unexpected serious adverse event (SUSAR), the sponsor should report it after awareness of it for the first time as soon as possible and no later than 15 natural days.

3) For other information on the potential serious safety risks, the sponsor should report it after awareness of it for the first time as soon as possible and no later

than 15 natural days.

The above is the procedure for SUSAR reporting in China, each study site should report it according to local regulatory requirement.

11.8. Safety reporting

Investigators and the sponsor should provide data on SAE and safety risk evaluation report according to local/national ethics committee and regulatory requirements in time.

11.9. Treatment and management of adverse events

Investigators should establish contingency plan for SAE management in clinical trials, train all the relevant staff, take measures to be aware of any clinically significant disease/event after vaccination, and urge the subjects to go to the designated hospital for appropriate treatment in time. The drugs for treatment of AE should be recorded in the subject's original record and eCRF.

In case of disagreement and dispute in management of adverse events, investigators have the obligation to cooperate with the sponsor to deal with it and assist subjects in medical assessment.

The sponsor has the obligation and responsibility to ensure the safety of subjects unconditionally, and provide humane care and compensation for the subjects with AEs related with the Investigational product during participation in the clinical trial.

For the AEs that are still ongoing at the termination or end of visit, investigators should pay more attention to them continuously, the AEs related with the vaccination should be followed up until they are resolved, and the follow-up of unrelated event, such as disease, can be discontinued upon acquisition of the diagnosis.

11.10. Pregnancy events

All pregnancy events occurred within 1 year from the first dose of vaccine to the full course of vaccination should be reported within 5 days after being informed and the investigators should fill in the "Pregnancy Report Form".

Investigators will closely follow the pregnant subjects, and obtain information about pregnancy outcomes (for example, details of delivery and newborn situations or termination of pregnancy), and update the "Pregnancy Report Form." The follow up visits for the will last for one year, and whether to continue the follow-up visit will be determined according to the non-clinical results and the one-year observation results.

Pregnancy itself is not considered an SAE, but any complications during pregnancy will be considered as AE and in some cases can be considered as SAE, such as spontaneous abortion, stillborn foetus, stillbirth and congenital abnormalities of infants. When no abnormalities are found in the fetus, induced abortion due to the

mother's personal decision is not considered AE.

12. Study Support Teams and Their Responsibilities

12.1. Endpoint Assessment Committee (EAC)

EAC is established to confirm the cases of COVID-19 occurring during study period and judge according to the grading criteria for COVID-19 stipulated in study protocol.

Description of responsibilities:

(1) Able to review, approve and complete related work in a timely manner in accordance with the EAC charter;

(2) The chairman is responsible for supervising whether the review of the endpoint event is carried out in accordance with the trial protocol; shall attend all meetings; record (only all the review results) in the summary report form and sign; responsible for checking meeting minutes and signing; coordinate and reach a consensus, and communicate the views to the sponsor;

(3) In case of any endpoint event, objectively evaluate the endpoint event according to the unified definition standards, combined with clinical expertise and relevant contents in the protocol to determine whether it conforms to the definition standards;

(4) Review the description of all events and check the source documents of each event. Necessary relevant information in the source documents obtained by the committee members have to be masked to ensure that blind review is achieved;

(5) During the independent review process, the committee members can request to provide the required source documents, and review relevant clinical data (i.e., lung imaging, death certificate, hospitalization records, etc.) before making the final decision;

(6) Members reach a consensus on the independent review, and the review results will be announced at the meeting and the chairman will sign for confirmation in the summary report form. If the independent review opinions of the committee members cannot reach a consensus, review meetings (regular meetings or ad hoc meetings) will be held as necessary to discuss them. If there are still disagreements after the discussion, voting shall be performed. Voting must also follow certain rules;

(7) If the committee members need additional source documents during the review meeting, they should be recorded in the meeting minutes and make

supplementary application after the meeting;

(8) The EAC management team needs to cooperate with the data management department to complete the data question proposal and answer for the review results, which is different from the general clinical trial question answering process;

(9) After completing evaluation, formulate the final Master Binder.

12.2. Data and Safety Monitoring Board (DSMB)

An independent DSMB is established, which is composed of experts possessing the necessary knowledge of clinical trial. Before each meeting, data report is received by DSMB. If the preset conditions are achieved in the study, a formal interim analysis will be made by DSMB. All data reviewed by DSMB are strictly kept secret. In the chapters of DSMB, the responsibility of DSMB, the number of interim reports and the way of operation are stipulated. The interim report is written by independent statisticians. All suggestions of DSMB are conveyed to field PI. Written summary report of DSMB and applicable suggestions are submitted by field PI to local/national ethics committee and other applicable institutions.

Description of responsibilities:

(1) Verify, approve and complete relevant work in time according to the stipulations in chapters of DSMB;

(2) Verify study protocol, verify efficacy/safety data and raise suggestions for revision of monitoring plan;

(3) Execute the verification of data at unblind state according to the monitoring plan. The efficacy/safety data are exhibited at unblind state through the information of actual study grouping (including: true name of two groups).

(4) If the conditions are allowed, the factors beyond study are explored, such as scientific or therapeutic progress possibly causing a problem in safety of subjects or ethics of study.

(5) Participate in discussion of DSMB, and vote for suggestions of DSMB when necessary;

(6) Suggest the sponsor for other modifications during the course of study and after the termination of study based on the observed data;

(7) If severe, critical or fatal case occurr after the subject is infected with SARS-COV-2 during study period, a special investigation should be conducted. The DSMB shall conduct an analysis based on the findings of the specific

investigation. If the analysis suggests that ADE/VED exists, the DSMB shall convene an emergency meeting to assess the risk of ADE/VED in the entire trial and immediately report it to the institutional review board (IRB) / independent ethics committee (IEC) and relevant regulatory authorities.

13. Statistical Analysis

Besides study protocol, an independent statistical analytical plan is formulated, which illustrates the technical details of statistical analysis in more detailed way. The SAP will be finalized before database lock.

13.1. Research hypothesis

Primary study hypothesis

The lower limit of the 95% confidence interval (CI) for protection against COVID-19 of any severity is greater than 30%, compared with placebo, at least 14 days after full course of immunization.

13.2. Sample size considerations

13.2.1 Sample size calculation based on efficacy study

A large-scale validatory clinical study is made to evaluate the efficacy of Investigational product against COVID-19 in the population of ≥18 weeks old. By assuming that the occurrence rate of COVID-19 of any severity is 1% during the study period, power of test is calculated. According to the study plan, when COVID-19 of any severity is observed in 50% of subjects, an interim analysis is made; and total error of type I is controlled within 5% (two-sided) through the consumption function of O'Brien-Fleming. In order to study 60% efficacy of Investigational product (lower limit of 95% CI is >30%), a total of 155 COVID-19 cases of any severity and 22144 subjects (11072 cases each group) are required to make the power of test reach 90%. The number of events is calculated through exact conditional method according to the large-sample hypothesis of Poisson distribution of Chan and Bohidar. Therefore, by overall considering the conditions of dropout, protocol deviation and incompliance, 14000 subjects will be recruited into each group according to study plan.

The number of cases in some countries and centers is unpredictable; the morbidity rate of COVID-19 varies with country; and the number of COVID-19 cases is verified at blind state during the study course. Therefore, during the study course, the number of subjects to be enrolled can increase with the change of morbidity rate

in different regions; but the required number of COVID-19 cases of any severity can not be changed, so that total error of type I during study course is not expanded.

13.2.2 Sample size calculation based on immunogenicity bridging study

Set a non-inferior effect value of 0.67, the inspection level α of 0.025 unilateral, and a master degree of 90%, and assume that the GMT of anti-SARS-COV-2 neutralizing antibody in people in China and outside China is the same, the standard deviation of the antibody titer after logarithmic conversion is 0.55, and the distribution ratio of the two groups of samples is 1:1. Using PASS 15, the minimum sample size of each investigational vaccine group in China and outside China was 207. Considering factors such as shedding and age distribution, 1000 subjects were planned to be enrolled in each experimental vaccine group in China and outside China (750 subjects aged 18-59 and 250 subjects aged 60 and above).Therefore, a total of 2,000 patients are to be enrolled, including 1,000 in China and 1,000 outside China (1,000 subject outside China will be immunogenicity subgroup of the efficacy study cohort, which will also participate in the efficacy evaluation). There will be 1000 in the investigational vaccine group and 1000 in the placebo group.

To sum up, the total sample size of the Phase III clinical trial will be 29,000.

Because of the unpredictable number of cases in some countries and centers and the incidence rate in various countries, the number of COVID-19 cases is checked blindly during the test. The number of subjects planned to enter the group can be increased during the trial according to the incidence rate of different regions, but the number of COVID-19 cases of any severity required can not be changed, so the total type I error will not be inflated.

13.3. Analysis Sets

✧ **Efficacy analysis sets**

Full Analysis Set for Efficacy (E-FAS): It includes all subjects observing the principle for intent-to-treatment (ITT), entering the stage of randomization, completing the inoculation of vaccine for at least one dosing, and receiving at least one follow-up of case monitoring after the inoculation.

Modified Full Analysis Set for Efficacy (E-mFAS): It is a subset of E-FAS; it includes all subjects completing the whole-course inoculation of vaccine for two dosings and receiving at least one follow-up of case monitoring from 14 days after the whole-course inoculation.

Per-Protocol Set for Efficacy (E-PPS): It includes all subjects conforming to

inclusion/exclusion criteria, entering the stage of randomization, completing the whole-course inoculation of vaccine for two dosings, receiving at least one follow-up of case monitoring from 14 days after the whole-course inoculation and not seriously violating study protocol.

In the E-mFAS and E-PPS, the cases are calculated from 14 days after the whole-course inoculation, which is mainly applied to evaluate primary efficacy of vaccine; in the E-FAS and E-mFAS1, the cases are calculated after the inoculation of vaccine for first dosing.

✧ **Immunogenicity analysis sets**

Full Analysis Set for Immunogenicity (I-FAS) : It includes all subjects observing the principle for intent-to-treatment (ITT), having completed randomization and received at least one dose of vaccine and having valid pre-immunization immunogenicity result.

Per-Protocol Set for Immunogenicity (I-PPS): It includes all subjects conforming to the inclusion and exclusion criteria, having completed full course of vaccination of vaccine and having valid immunogenicity results of both pre-immunization and 14 days after full course of vaccination.

Immune Persistence Set (IPS): It includes all subjects having completed blood sample collection for immune persistence evaluation 6 months after full course of vaccination and having valid antibody data.

I-FAS and I-PPS are applied for immunogenicity analysis and IPS is appiled for immune persistence analysis.

✧ **Safety analysis sets**

Safety Set (SS): It includes all subjects having received at least one dose of Investigational product.

✧ The safety analysis sets will also define the first dose safety analysis set, the second dose safety analysis set and the third dose vaccine safety analysis set. The first dose safety analysis set includes subjects who have completed the first dose of vaccine for post-dose safety analysis; the second dose safety analysis set includes subjects who have completed the second dose of vaccine for post-dose safety analysis; the third dose safety analysis set includes subjects who have completed the third dose of vaccine for post-dose safety analysis.

In efficacy and immunogenicity analysis, all subjects will be analyzed according to the group they are randomly assigned to; all safety data will be analyzed according to

their actual investigational group.

All analysis sets will be discussed by the principal investigator, the sponsor, the statistician and the data manager during a blind data review prior to database locking.

13.4. Statistical analysis method

13.4.1. General principle

Measurement data will be statistically described with mean, median, standard deviation, maximum and minimum; enumeration data or ranked data will be described with frequency and rate.

SAS Version 9.4 or above statistical software will be used for all the statistical analyses.

13.4.2. Study completion and demographic characteristics

The number of subjects screened, enrolled in each group and completing the study, as well as the number of subjects in each analysis set will be summarized, respectively, the drop-out subjects and reasons for drop-out will be analyzed. The demographic characteristics of the subjects in each group will be statistically described.

13.4.3. Evaluation of efficacy

13.4.3.1.Evaluation of primary efficacy

The person-year incidence of any severity of COVID-19 diagnosed and its 95% confidential interval will be calculated in the vaccine group and placebo group 14 days after completion of the full course of vaccination, Poisson regression model is used to carry out statistical comparison of the intergroup difference, the vaccine protection rate and its 95% confidential interval based on person-year incidence will be estimated based on the model. In Poisson regression model, the number of patients is the dependent variable, center, age group (18~59 years, 60 years and above) and group are fixed effects, person-year exposure of subjects is offset, log link function is used. In case the person-year incidence is close to 0 in vaccine group or placebo group, the exact method will be used to calculate the 95% confidential interval of the protection rate based on person-year incidence.

Where, the person-year incidence = (number of patients/person-year exposure of subjects) ×100%. In the calculation of person-year exposure, the start time is 14 days after full course of vaccination, the time of termination of any severity of COVID-19 cases is the time at the first discovery of the case, the time of termination of other subjects is the last follow-up time for observation of efficacy. Vaccine protection rate = 1- (person-year incidence in vaccine group / person-year incidence in placebo group).

The efficacy for any severity of COVID-19 diagnosed 14 days after full course of vaccination will be evaluated based on E-mFAS and PPS.

13.4.3.2 Evaluation of secondary efficacy

In addition, evaluation of the efficacy for severe and critical COVID-19 14 days after full course of vaccination as well as the evaluation of the efficacy of at least one dose of vaccination for any severity of COVID-19 based on FAS use the same statistical analysis method with that for evaluation of primary efficacy.

13.4.4. Immunogenicity evaluation

13.4.4.1. Immunogenicity evaluation in immunogenicity bridging study

The GMT of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group in immunogenicity bridging study is statistically compared in the analysis of covariance model fitted after logarithmic transformation, which uses the GMT of SARS-COV-2 neutralizing antibody following logarithmic transformation 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group as the dependent variable, logarithmic conversion result of SARS-COV-2 neutralizing antibody prior to immunization as covariate, region (including China and outside China) and age group (18~59 years, 60 years and above) as fixed effect, GMT of SARS-COV-2 neutralizing antibody following logarithmic transformation 14 days after full course of vaccination in the pre-immunization negative population as well as the least squares means of intergroup GMT ratio and their 95% confidential interval are calculated for each region based on the model; and upon inverse logarithmic transformation, GMT of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group as well as the least squares means of intergroup GMT ratio and their 95% confidential interval are calculated for each region. The lower limit of two-sided 95% confidential interval of the GMT ratio of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in domestic investigational vaccine group versus oversea investigational vaccine group is calculated, and the immunogenicity will be considered as non-inferior in the investigational vaccine group in China to that outside China if it is >0.67.

The positive rate and positive conversion rate of SARS-COV-2 neutralizing antibody and IgG antibody are calculated in the total population, pre-immunization negative population and pre-immunization positive population at each time point post immunization for different regions (China and outside China), in the investigational vaccine group in immunogenicity bridging study, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test / Fisher exact probability method is used for the statistical test of the difference across different regions.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI (GMT growth multiple) of SARS-COV-2 neutralizing

antibody and IgG antibody 14 days after full course of vaccination in the total population, pre-immunization negative population and pre-immunization positive population for different regions (China and outside China), in the investigational vaccine group in immunogenicity bridging study, respectively, and paired t test following logarithmic transformation is used for statistical testing of the difference across different regions.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and IgG antibody prior to vaccination and 14 days after full course of vaccination is plotted for different regions (including China and outside China).

13.4.4.2. Evaluation of immunogenicity in immunogenicity subgroup

The positive rate and positive conversion rate of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody in the total population, pre-immunization negative population and pre-immunization positive population at each time point post immunization are calculated for vaccine group and placebo group in immunogenicity subgroup, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test /Fisher exact probability method is used for statistical test of the difference between vaccine group and placebo group.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody in the total population, pre-immunization negative population and pre-immunization positive population in vaccine group and placebo group in immunogenicity subgroup, respectively, and paired t test following logarithmic transformation is used for statistical testing of the difference across different regions.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody prior to vaccination and 14 days after full course of vaccination is plotted for vaccine group and placebo group in immunogenicity subgroup, respectively.

13.4.4.3. Evaluation of immune persistence

The positive rate of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization is calculated in vaccine group and placebo group in immunogenicity subgroup, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test /Fisher exact probability method is used for statistical test of the difference between vaccine group and placebo group.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization in vaccine group and placebo group in immunogenicity subgroup, respectively.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization is plotted for vaccine group and placebo group in immunogenicity subgroup, respectively.

13.4.5. Safety evaluation

MedDRA is used for medical coding of adverse events and serious adverse events, which will be statistically summarized by system organ class (SOC) and preferred term (PT). In addition, the solicited adverse events will be statistically summarized by adverse event at injection site and non-injection site (systemic) as specified in the protocol. Treatment emergent adverse events (TEAE) following vaccination are mainly statistically analyzed in this study, and those occurred prior to vaccination will be presented in a form of list. Unless otherwise noted, the adverse events in the following text are TEAEs.

Number of AEs, number of subjects and incidence of all the adverse events, adverse events related with the Investigational product, adverse events unrelated with the Investigational product, grade 3 and above adverse events, grade 3 and above adverse events related with the Investigational product are calculated in the investigational vaccine group and placebo group, respectively, Fisher exact probability method is used for statistical comparison of the difference in the incidence of the above adverse events between the two groups. The time to occurrence of adverse events, dose time and severity are statistically described.

Adverse events following each dose time of vaccination are statistically analyzed, respectively. Analysis of adverse events following each dose time will be performed on the safety set of each dose time.

List of adverse events related and unrelated with the Investigational product will be presented.

Number of AEs, number of subjects and incidence of all the serious adverse events, serious adverse events related with the Investigational product and serious adverse events unrelated with the Investigational product are calculated in investigational vaccine group and placebo group, respectively, Fisher exact probability method is used for statistical comparison of the difference in the incidence of the above adverse events between the two groups. List of serious adverse events will be presented.

13.5. Interim analysis (IA)

This is a case-driven study. If 78 cases of COVID-19 (50%) are observed during the study course, an interim analysis will be made. Total error of type I is controlled through the consumption function of O'Brien-Fleming. When 78 cases of COVID-19 of any severity are observed and interim analysis is made, nominalα for IA is $\alpha 1 = 0.003$ (two-sided) as calculated through the consumption function of O'Brien-Fleming. In other words, if P value of IA is <0.003, null hypothesis is

refused, and the study is terminated in advance; otherwise, the study is continued until 155 cases of COVID-19 are observed for final analysis (FA). Nominalα for FA is α2 = 0.049 (two-sided). During the study course, nominal significance level of IA and FA is estimated through the consumption function of O'Brien-Fleming according to the actual number of observed cases of COVID-19 at IA. If there are more than 78 actual cases of COVID-19 at IA, α is assigned again through the method of LM_O'Brien-Fleming.

13.6. Multiplicity

The O'Brien Fleming consumption function is used to control the total type I errors within 5% during the interim analysis. Please refer to the interim analysis section for details.

13.7. Statistical analysis strategy

This study will be able to conduct statistical analysis for NMPA declaration when the above sufficient number of events is reached; after 1-year observation and follow-up, the protective effect of vaccine will be further evaluated based on all collected cases.

14. Data management

14.1. eCRF design

eCRF will be designed in accordance with the study procedures and flow chart in the protocol, needs to be reviewed jointly by project manager, data administrator, statistician and protocol writer after formation of the draft, meet the protocol and comply with relevant laws and regulations, and the process of version control needs to be completely recorded.

14.2. Guideline on eCRF filling

The guideline on eCRF filling is the detailed description on filling in each page and each data point of eCRF according to the study protocol. Acquisition of eCRF and guideline on its filling at the clinical trial center needs to be guaranteed prior to enrollment of subject, and relevant staff at the clinical trial center will be trained on the protocol, eCRF filling and data submission process, which needs to be archived for record.

14.3. Note to eCRF

Note to eCRF is the marking to blank eCRF, record of the place of each data item in eCRF as well as the variable name and code in the database. All the data items in eCRF need to be marked. DM review is required.

14.4. Database design

The database should be established in accordance with the name of dataset, name of variable, type and length of variable in noted eCRF, and comply with the structure and configuration of standard database as much as possible. After completion of establishment of database, the database should be tested, the database test report needs to be issued and signed by the person in charge of data management for confirmation.

14.5. Permission assignment

According to different roles, accounts will be created by system administrator separately, and different permissions will be granted.

14.6. eCRF filling

The study personnel need to collect subject's data according to the requirements in the study protocol, and fill the data in eCRF in an accurate, timely, complete and standard manner according to the guideline on filling, based on the original materials. Modification of the data on eCRF must comply with the standard operating procedures, and the modification traces need to be maintained.

14.7. Transmission and resolution of questions

Data Management (DM) will list a detailed data verification plan, which will be signed by data administrator, data manager and the sponsor for confirmation upon review and no objection by the sponsor, medical staff, statistician and project manager. After entry of data in EDC, the system will verify the data in accordance with the Edit Check established in the data verification plan and send queries automatically for all the questionable data; a manual query will be sent through EDC for the data for which the system can not be set to send query, the entry clerk or investigator will confirm and answer manual queries and system queries, and modify the wrong data when necessary, until the query is solved. If the answer can not solve query, a query can be made on this data point again by data administrator and clinical monitor, all the traces will be kept in EDC database.

14.8. Data modification and review

After the data are verified by data-entry clerk or investigator, the data can be modified, the modified data need to be prompted in the system and the reason for modification should be shown in the system. Investigators can finally verify all data.

14.9. Medical coding

Adverse events collected in clinical trials will be encoded using standard dictionary. MedDRA is the standard dictionary commonly used. The dictionary and version used for coding should be clearly recorded for the data set coded.

14.10. Comparison of SAE consistency

All the data points related with SAE in the database will be compared with the data

points in PV (Pharmacovigilance) system using program, inconsistent data need to be communicated with PV personnel, until no difference in the data.

14.11. Data review meeting

Before locking of database, the draft of data review report and all the data lists will be well prepared, the database will be finally reviewed by the sponsor, investigators, data administrator and statisticians together, and division of statistical analysis population, verification of serious adverse event report and treatment record, verification of ADE/VED report and treatment record will be carried out according to the clinical study protocol, the data review report and population division plan need to be finalized after the data review meeting.

14.12. Lock and unlock of database

Lock of database is one important milestone during clinical study. The process and time of locking should be clearly documented, locking is the cancellation of the permission to edit the database, any unauthorized account can not manipulate the database. If there is any modification after lock of database, it needs to be applied and can be executed only after discussion and signature by the sponsor, investigators, statisticians, clinical monitors and data administrator for confirmation, and the reason for unlocking needs to be recorded carefully.

15. Maintenance and Management of Material

15.1. Management of original material

The informed consent form, vaccination and follow-up record book, diary card, contact card, SAE report form and other original materials are important basis for traceability of clinical trials, should be recorded in a timely, accurate, complete, standard and authentic manner, and properly maintained at the study site.

The study data will be entered in EDC by authorized and specially trained investigators in accordance with the original materials, can not be changed ad arbitrium during the entry, and should be modified in accordance with the guideline on filling for wrong entry. In order to ensure the authenticity and reliability of the clinical trial data, EDC will be reviewed by monitors and investigators jointly. All the materials will be statistically processed by the unit in charge of the clinical trial or the statistician entrusted by the sponsor upon signature of investigators.

15.2. Study material

The sponsor and study party will provide clinical trial materials in accordance with the regulations on drug registration management and GCP provisions.

Investigator's folder will be arranged as required by GCP and maintained at the study site. The study site is responsible for arranging and summary of the materials

delivered to the sponsor. Materials recording true information of subjects, such as screening registration form, informed consent form, vaccination and follow-up record book, diary card, contact card, subject's medical record, will be sealed at the study site, the coordinators in charge of the institutions will check and hand them over with the on-site archivist, both parties will sign the deposit agreement or memorandum.

File management will be carried out according to SOP, the identification label, including name of project, date of completion, sponsor and storage period, will be well prepared, insect-proof, moisture-proof, fire-proof and anti-theft measures will be taken. Use and access to the materials of this project is only limited to relevant staff in the project, the personnel from the sponsor (including clinical monitors) and inspectors from regulatory authorities. All the materials for application of drug registration will be maintained until 5 years after approval of the vaccine, the sponsor will be informed on the due date, the materials can not be disposed by anyone without authorization, prior to acquisition of the written notification from the sponsor.

16. Quality Control and Quality Assurance Procedures

This study will be carried out in accordance with relevant regulations and standard operating procedures.

The study will be conducted in accordance with this protocol, the ICH GCP) and any applicable regulatory requirements. Biological samples will be processed, stored and transported in accordance with SOP.

Data validation will be performed to identify errors or discrepancies to ensure the integrity, validity and accuracy of the data.

17. Ethical and Legal Considerations

17.1 Declaration of Helsinki

The investigators assure that this study will be carried out in accordance with the principles from Declaration of Helsinki.

17.2 Good Clinical Practice

The investigators assure that this study will be performed in compliance with the Good Clinical Practice.

17.3 Review of the clinical trial

The ethics committee shall review the scientific and ethical rationality of the drug clinical trial project in order to ensure the dignity, safety and rights of the subjects, promote the scientific and healthy development of the drug clinical trial, and enhance the public's trust and support for the drug clinical trial.

The ethnics committee may review the test protocol, informed consent, recruitment

materials and other written materials provided to the subjects. The revision of the protocol shall be negotiated with the sponsors. The content of the informed consent form may be accepted by the ethnics committee if it does not violate the protocol and conforms to the local actual conditions.

17.4 Confidentiality of subject information

The study teams will keep the confidentiality of subject information. In CRF, initials of name of subjects can be indicated; in other study documents and electronic database, the identity of subjects can be indicated only through the study No. of subjects. All documents are stored in safe way, and can be accessed only by study team and authorized persons.

17.5 Compensation

The subjects will not be paid for taking part in this study. The expenses of subjects incurred during study period is reimbursed according to the local applicable guidelines and the policies of ethics committee.

17.6 Report

From the approval date of study, annual progress report is annually submitted by PI to all applicable ethics committees. In addition, after the completion of study, a study termination report is submitted by PI to all applicable ethics committees.

18. Insurance

An insurance of clinical trial is arranged for subjects. If a harm related to clinical trial occurs in the subjects during the course of clinical trial, the corresponding compensation will be made.

19. Paper Publishing

After the conclusion of the trial, the research unit may publish the summary report or research results involving the clinical trial in the form of a paper after obtaining the written authorization of the sponsor, and the researchers of the research unit and the technical cooperation unit (pharmacodynamic evaluation) shall have the right of authorship of the paper. Negative or inconclusive research results should be published or made public in the same way as positive results.

20. References

1. Novel Coronavirus Infection Diagnosis and Treatment Plan (trial version 7), China National Health Commission, March 3rd, 2020.
2. Zumla A, Chan JFW, Azhar EI, et al. Coronaviruses-drug discovery and therapeutic options［J］. Nat Rev Drug Discov, 2016,15(5):327-347．.
3. Graham RL, Donaldson EF, Baric RS. A decade after SARS:strategies for controlling emerging Coronaviruses［J］. Nat RevMicrobiol, 2013, 11(12):836-848.
4. Li W, Moore MJ, Natalya Vasilieva, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS Coronavirus ［J］.Nature, 2003, 426(6965):450-454
5. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human Coronavirus-EMC［J］.Nature, 2013, 495(7440):251-254.
6. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 CoronavirusNovel Coronavirus: implications for virus origins and receptor binding［J］. Lancet, 2020. DOI: 10.1016/S0140-6736(20)30251-8
7. Guideline on the Classification of Adverse Events in Clinical Trials of Preventive Vaccines, NMPA, December 31, 2019
8. Good Clinical Practice of Pharmaceutical Products (GCP), NMPA, April 23, 2020.
9. ICH E6, GCP, 1996

21. Annex A: Subject Study Workflow

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9
Time of visit (days)	**D-7~0**	**D0**	**V2+8**	**V2+30**	**V4+8**	**V4+14**	**V4+30**	**V4+180**	**V3+360**
Window period (days)	**/**	**/**	**/**	**+7**	**/**	**+7**	**/**	**+30**	**/**
Informed consent	X								
Urine pregnancy		X							
Demographic information	X								
Medical history and allergy history	X	X							
Axillary temperature	X	X		X					
SARS-COV-2 RT-PCR	X								
SARS-COV-2 IgG and IgM	X								
Verification of inclusion/exclusion criteria	X	X							
Randomization		X							
Vaccination		X		X					
Observation of 30 minutes after vaccination		X		X					

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9
Time of visit (days)	**D-7~0**	**D0**	**V2+8**	**V2+30**	**V4+8**	**V4+14**	**V4+30**	**V4+180**	**V3+360**
Window period (days)	/	/	/	**+7**	/	**+7**	/	**+30**	/
Dispensing the thermometers, dipperstick, diary cards and implementing the trainings [3]		X		X					
Recovery of diary cards, and distribution of contact cards			X		X				
Recovery of contact cards				X			X		
Concomitant medications[4]	X	X	X	X	X	X	X	X	X
Blood sampling for immunological assay[1]		X				X		X	
Report serious adverse events		X	X	X	X	X	X	X	X
Report pregnancy event		X	X	X	X	X	X	X	X
Monitoring of COVID-19 cases[2]		X	X	X	X	X	X	X	X

1. The collection of blood sample for immunological test is only applicable for 750 subjects of 18~59 years old outside China, 250 subjects of ≥60 years old outside China and all subjects in China.

2. If suspected cases of COVID-19 are found after the inoculation for the first dosing, throat swab is collected for RT-PCR test.

3. At Visit 1, the thermometer, straight ruler and diary card is granted; at Visit 3, the diary card is granted.

4. Only the concomitant medications for treating SAE and pregnant complications are collected from 30 days to 12 months after the whole-course inoculation of vaccine.

22. Annex B: Adverse Events Grading Scale

Clinical observation indicators (Table 1-3)

Table 1. Injection-site (local) Adverse Events Grading Scale

Symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Pain and tenderness (optional; and tenderness is applicable for the subjects unable to express the pain by themselves)				
Pain	No or slight influence on the activity of limbs	Influencing the limb movement	Influencing the daily life	Loss of basic ability for self care, or causing a hospitalization
Tenderness	Resistance and withdrawal at a contact or touch	Comfortable crying at a contact or touch	Uncomfortable continuous crying	Requiring the emergency care or hospitalization
Induration*, swelling (optional) ** #				
> 14 years old	Diameter 2.5~or area 6.25~2 and with no or slight influence on daily life	Diameter 5~ or area 25~2 or influence on daily life	Diameter ≥10 cm or area ≥100 cm2or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious influence on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue
Rash*, redness (optional) ** #				
> 14 years old	Diameter 2.5~5 or area 6.25~2 and with no or slight influence on daily life	Diameter 5~ or area 25~2 or with an influence on daily life	Diameter ≥10 cm or area ≥100 cm cm cm2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious influence on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue

Symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Others				
Pruritus	Pruritus at the site of inoculation, which is relieved spontaneously or within 48 hours after the treatment.	Pruritus at the site of inoculation, which is not relieved within 48 hours after the treatment.	Influencing the daily life	NA
Cellulitis	NA	Requiring the non-injection treatment (e.g., oral administration of antibiotic, antifungal, and antiviral drugs)	Required treatment of intravenous injection (such as intravenous injection of antibacterial, antifungal and antiviral drugs)	Pyemia/sepsis, or tissue necrosis

Note: *The diameter is directly measured for grading evaluation, and the progress/change of measurement results is also recorded.

**Maximum measured diameter or area is adopted.

The induration, swelling, rash and redness are evaluated and graded according to the functional grade and the actual measurement results through the indices of higher grade.

Table 2. Vital Signs Grading Scale

Signs	Grade 1	Grade 2	Grade 3	Grade 4
Fever* [axillary temperature (°C)]				
> 14 years old	37.3~<38.0	38.0~<38.5	38.5~<39.5	≥39.5, lasting for over 3 days
Electrocardiogram PR interval prolonged or atrioventricular block (optional)				
> 16 years old	PR interval: 0.21s - <0.25s	PR interval ≥0.25 seconds or type I atrioventricular block of Degree 2	Type II atrioventricular block of Degree 2 or ventricular interval ≥3 seconds	Complete atrioventricular block
Heart rate				
Tachycardia (times/min)	101~115	116~130	>130	Arrhythmia requiring an emergency treatment or a hospitalization
Bradycardia (times/min)	50~54	45~49	<45	Arrhythmia requiring an emergency treatment or a hospitalization
Blood pressure				
Hypertension (mmHg)				
≥18 years old	Systolic pressure 140~or diastolic pressure 90~<100	Systolic pressure ≥160~or diastolic pressure ≥100~<110	Systolic pressure ≥180or diastolic pressure ≥110	Occurrence of life-jeopardizing complications not diagnosed previously (such as malignant hypertension) or causing a hospitalization
Hypotension (systolic blood pressure) (mmHg)	85~<89	80~<85	<80	Shock or causing a hospitalization
Respiratory frequency (times/min)	17~20	21~25	>25	Requiring tracheal intubation

Note: * In China, axillary temperature is generally adopted, which is converted into oral temperature and anal temperature when necessary. Generally, the formula for conversion is adopted: Oral temperature = Axillary temperature + 0.2°C; Anal temperature = Axillary temperature + (0.3~0.5°C). When a continuous high fever

occurs, the reason for high fever should be determined as soon as possible.

Table 3: Grade of adverse events in whole body (except the injection site)

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal system				
Diarrhea	Mild or transient, 3~4 times a day, abnormal property of stool, or mild diarrhea continuously for <1 week	Moderate or continuous, 5~7 times a day, abnormal property of stool, or diarrhea continuously for >1 weeks	>7 times a day, abnormal property of stool, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, necessary for intravenous infusion at >2 L	Hypotensive shock, requiring hospitalization
Constipation*	Necessary for stool softeners and diet adjustment	Necessary for laxatives	Refractory constipation necessary for manual defecation or enema application	Toxic megacolon or intestinal obstruction
Swallowing difficult	Mild discomfort when swallowing	Dietary restrictions	Very limited in diet and talk; unable to take solid food	Unable to take liquid food; necessary for intravenous infusion of nutrients
Anorexia	Decreased appetite but no reduction of food intake.	Inappetence, decrease of food intake, no obvious decrease of body weight	Decreased appetite with significant weight loss	Requiring the intervention measures (e.g., gastric tube feeding, and parenteral nutrition)
Vomiting	1-2 times per 24 hours and no influence on movements	3-5 times per 24 hours or limited movements	>6 times within 24 hours or necessary for intravenous infusion	Necessary for hospitalization or nutritional support through other channels due to the hypotensive shock

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	Transient (or intermittent and basically normal intake of food	Continuous nausea causing a decrease of food intake (24~48 hours)	Continuous nausea causing a hardly intake of food (>48 hours) or necessary for intravenous fluid infusion	Life-threatening (such as hypotensive shock)
Musculoskeletal and connective tissues disorders				
Pain muscle (non-injection site)	Not influencing the daily life	Slightly influencing the daily life	Serious muscular pain with a serious influence daily life.	Emergency care or hospitalization
Arthritis	Mild pain with inflammation, erythema, or joint swelling; not limiting the functions	Moderate pain with inflammation, erythema, or joint swelling; limiting the functions, but not influencing the daily life	Severe pain with inflammation, erythema or joint swelling; influencing the daily life	Permanent and/or disabling injury of joint
Pain joint	Mild pain, but not limiting the functions	Moderate pain; necessary for analgesics and/or causing a dysfunction, but not influencing the daily life	Serious pain; necessary for analgesics and/or influencing the daily life	Disability pain
Nervous system				
Headache	Not influencing the daily life, requiring no treatment	Transient, slightly influencing the daily life, and possibly requiring a treatment or intervention	Serious influence on daily life, requiring a treatment or intervention	Refractory, requiring an emergency treatment or a hospitalization
Syncope	Near-syncope without unconsciousness (such as pre-syncope)	Unconsciousness, but unnecessary for treatment	Loss of consciousness, requiring treatment or hospitalization	NA
Newly occurring convulsions				

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
≥18 years old	NA	NA	Convulsions with 1-3 times	Convulsion for a long time and for several times (such as status convulsivus) or at uncontrollable state (such as refractory epilepsy)
Respiratory system				
Cough	Transient, requiring no treatment	Constant coughing, with effective treatment	Paroxysmal cough uncontrollable through the treatment	Emergency care or hospitalization
Acute bronchospasm	Transient; unnecessary for treatment; FEV1% 70%~80%	Necessary for treatment; resolvable through the bronchodilators; FEV1% 50%~70%	Unresolvable through the bronchodilators; FEV1% 25%~50% or continuous sinking of intercostal area	Cyanosis; FEV1% <25%; or necessary for intubation
Dyspnea	Exercise dyspnoea	Dyspnea in normal activities	Dyspnea when rest	Dyspnea, necessary for oxygen inhalation, hospitalization or assisted respiration
Skin and subcutaneous tissue disorders				
Non-injection site pruritus (no skin injury)	Mild pruritus, with no or slight influence on daily life	Pruritus, influencing the daily life	Pruritus causing unable activity of daily life	NA
Skin and mucosa abnormality	Erythema/pruritus/color changed	Diffusive rash, maculopapule, dry skin, desquamation	Herpes, exudation, desquamation /ulcer	Exfoliative dermatitis involving mucosa, or erythema multiforme, or suspected Stevens-Johnsons syndrome
Nervous system				
Insomnia*	Mild insomnia, with no or slight influence on daily life.	Moderate sleeping difficulty, influencing the daily life	Serious insomnia, seriously influencing the daily life, requiring a treatment or hospitalization	NA

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Irritation or suppression	Mild irritation or slight suppression	Irritability or somnolence	Uncomfortable or low response	NA
Mental disorder (including: anxiety, depression, mania and delirium) Symptoms should be reported in detail	Mild symptoms, unnecessary for hospital visit or behavior with no or slight influence on daily life.	With clinical symptoms, necessary for hospital visit or behavior influencing the daily life.	Necessary for hospital visit or behavior ability not supporting the daily life.	Tendency to injure the self or others or acute delirium or loss of basic ability for self care
Immune system				
Acute allergic reaction**	Localized urticaria (vesicle), unnecessary for treatment	Localized urticaria necessary for treatment or mild angioedema unnecessary for treatment	Extensive urticarial or angioedema necessary for treatment or mild bronchospasm	Allergic shock or life-jeopardizing bronchospasm or throat edema
Others				
Fatigue, asthenia	Not influencing the daily life	Influencing the daily life	Serious influence on daily life, unable to work	Emergency care or hospitalization
Non-injection site pain# (please note the site when reporting)	Mild pain, not or slightly influencing the daily life	Pain, influencing the daily life	Pain, with a failure in the daily life	Injuring/disabling pain, loss of basic ability for self care

Note: FEV1%: Forced expiratory volume in one second; (FEV1) / Forced vital capacity (FVC)

*: In the subjects with constipation and insomnia, an attention should be paid to the change before and after the inoculation.

**Type I hypersensitivity

#: Pains other than the pain at the site of inoculation (except muscular pain, joint pain and headache).

General Principles for the Grading of Other Adverse Events

The severity of adverse events not listed in the grading scale is evaluated according to the following criteria:

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild: for a short time (<48 hours) or mild discomfort, not influencing the daily life, unnecessary for treatment.	Moderate: Slight or middle restraint of activity, possibly requiring a hospital visit, unnecessary for treatment or only requiring a slight treatment.	Severe: significant movement limitation Restricted, necessary for hospital visit and treatment, possibly requiring a hospitalization	Critical: Possibly life-jeopardizing, serious restraint of activity, necessary for monitored treatment.	Death

REFERENCES

1. NMPA Guideline on the Classification of Adverse events in Clinical Trials of Preventive Vaccines. December 31, 2019

Study title: A Phase III Randomized, Double-blind, Placebo-controlled Clinical Trial in 18 Years of Age and Above to Determine the Safety and Efficacy of ZF2001, a Recombinant Novel Coronavirus Vaccine (CHO cell) for Prevention of COVID-19

Protocol number: LKM-2020-NCV-GJ01

Date / Version No.: Jun 12, 2021 / V1.3

Statement of Confidentiality

Statement and Signature Page of Sponsor

I, the undersigned, have reviewed the clinical trial protocol, and agree with the contents of the protocol. I will perform the relevant duties in strict compliance with the local laws, the Declaration of Helsinki, the GCP requirement and this protocol. I will provide copies of the protocol to every participant of the clinical trial from our company, and discuss the protocol and relevant information with them to ensure that they fully understand the investigational drug, understand how to conduct the trial, and ensure that the trial is carried out according to the protocol.

Sponsor: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.

Person in Charge: Shilong Yang

Signature: _____Date: _____

Statement and Signature Page of Principal Investigator

I, the undersigned, have reviewed the clinical trial protocol, and agree with the contents of the protocol. I will perform the relevant duties in strict compliance with the local laws, the Declaration of Helsinki, the GCP requirement and this protocol. I will provide copies of this protocol to every investigator who participated in the trial under my charge, and discuss the protocol and relevant information with them to ensure that they fully understand the investigational drug, and how to conduct the trial.

Clinical Trial Institutions: Hunan Provincial Center For Disease Control And Prevention

Principal Investigator (Print): Fangjun Li

Signature: _____Date: _____

Statement and Signature Page of Contract Research Organization

I, the undersigned, have reviewed the clinical trial protocol, and agree with the contents of the protocol. I will perform the clinical monitoring duties in strict compliance with the local laws, the Declaration of Helsinki, the GCP requirement and this protocol. I will keep all materials and information provided by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd according to the confidentiality requirements. When these materials and information are to be submitted to the Independent Ethics Committee (IEC), it must be indicated that these materials are confidential.

Contract Research Organization:

Person in Charge (Print)

Signature: _____Date: _____

Statement and Signature Page of Data Management and Statistics Company

I, the undersigned, have reviewed the clinical trial protocol, and agree with the contents of the protocol. I will perform the relevant duties in strict compliance with the local laws, the Declaration of Helsinki, the GCP requirement and this protocol. I will provide copies of this protocol to all relevant personnel participated in the clinical trial data management and report writing, and discuss the protocol and relevant information with them to ensure that they fully understand the protocol and how to carry out the data management and report writing.

Data Management and Statistics Company: Beijing Keytech Statistical Technology Co., Ltd

Person in Charge (In Print): Zhiwei Jiang

Signature: _____Date: _____

Study Team

Sponsor

Company Name	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd			
Project Leader	Shilong Yang			
Contact Address	No.93 Kexue Avenue, Hefei High Tech Industrial Development Zone, Anhui Province		Postcode	230088
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Investigator

Study Institution				
Principal Investigator				
Contact Address			Postcode	
Mobile Phone		E-mail		

Monitoring Company

Company Name	
Project	

Leader				
Address			Postcode	
Mobile Phone		E-mail		

Statistical Analysis Company

Company Name	Beijing Keytech Statistical Technology Co., Ltd			
Project Leader	Zhiwei Jiang			
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Mobile Phone	18618483152	E-mail	zhi.wei.jiang@ktstat.com	

Table of Contents

1. Synopsis

Study Title	A Phase III Randomized, Double-blind, Placebo-controlled Clinical Trial in 18 Years of Age and Above to Determine the Safety and Efficacy of ZF2001, a Recombinant Novel Coronavirus Vaccine (CHO Cell) for Prevention of COVID-19
Generic title	Phase III clinical trial of Recombinant Novel Coronavirus Vaccine (CHO cell)
Investigational product	**Investigational Vaccine:** Name: Recombinant Novel Coronavirus Vaccine (CHO cell) Main ingredients: NCP-RBD from SARS-COV-2 Spike protein, aluminum hydroxide adjuvant. Dosage Form: injection Strength: 0.5mL/vial. It contains 25 μg NCP-RBD protein Batch number: see the drug inspection report. **Placebo comparator:** Name: Placebo for Recombinant Novel Coronavirus Vaccine (CHO cell) Main ingredients: Aluminum hydroxide adjuvant Dosage Form: injection Strength: 0.5mL/vial. It contains 0.25mg Aluminum hydroxide adjuvant. Batch number: see the drug inspection report.
Instruction for use	(1) take Recombinant Novel Coronaviru (CHO cell) / placebo, shake it up and down for 5-10 times, and mix well. (2) After mixing, take all the liquid and inject intramuscularly into deltoid muscle of upper arm; (3) Follow the 0, 1, 2 month immunization schedule.
Indications	For prevention of Coronavirus Disease 2019 (COVID-19) caused by SARS-COV-2 infection

Study Population	Population aged 18 years and above
Research Institution	
Location of Study	Several study sites in China and outside of China
Study Objectives	**Primary objective:** To evaluate the efficacy and safety of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against any severity of COVID-19 in a population aged 18 years and above. **Secondary objectives:** 1. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against the severity of severe and above COVID-19 in a population aged 18 years and above. 2. To evaluate the immunogenicity and immune persistence of the Recombinant Novel Coronavirus Vaccine (CHO Cell) in a population aged 18 years and above. 3. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) as emergency vaccination against any severity of COVID-9 in a population aged 18 years and above. 4. To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against any severity of COVID-9 in populations of different age group (18-59 years vs. 60 years and above). **Exploratory objectives:** To explore the immunological surrogate variables of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against COVID-19 in a population aged 18 years and above.

Study Design

Overall design: A randomized, double-blind, placebo-controlled international multicenter clinical trial design will be adopted. A total of 29,000 subjects aged 18 years and above are planned to be recruited, including 750 subjects aged 18-59 years and 250 subjects aged 60 years and above in China; 21,000 subjects aged 18-59 years and 7,000 subjects aged 60 years and above will be recruited outside China. Safety and immunogenicity will be evaluated among the Chinese subjects, and efficacy, immunogenicity and safety will be evaluated among the subjects outside China. Among them, 750 subjects aged 18-59 and 250 subjects aged 60 and above from outside China and all subjects from China will be selected as the immunogenicity subgroup for immunogenicity bridging study. The efficacy study cohort will set a immunogenicity subgroup of 1000 subjects, of which 500 are given either the investigational vaccine or the placebo (subjects in immunogenicity subgroup participated in the efficacy evaluation at the same time). At the same time, a domestic immunogenicity study cohort will be set up, with a total of 1,000 subjects, of which 500 are given either the investigational vaccine or the placebo. The IgG levels of SARS-COV-2 neutralizing antibody and RBD protein binding antibody will be detected by blood sampling before vaccination, 14 days and 6 months after full course of vaccination to evaluate the immunogenicity and immune persistence.

Region	Age group	Immunogenicity evaluation	Safety evaluation	Efficacy evaluation	Immunization schedule
China	18 -59 yrs. (750 cases)	All subjects	All subjects	None	Months 0, 1, 2
	60 years and above (250 cases)				
Outside	18 -59 yrs. (21,000	750 cases	All subjects	All subjects	Months 0, 1,

China	cases)				2
	60 years and above (7,000 cases)	250 cases			

Study population:

A total of 29,000 subjects aged 18 years and above, including 28,000 subjects outside China and 1,000 subjects in China.

Study Plan and Implementation:

After signing the informed consent form, the volunteers aged 18 years and above will receive the relevant examinations after an inquiry by investigator of the medical history (including COVID-19 history), recent medication(vaccine) history, allergy history and concomitant medications, and demographic data collection by the investigators, including physical examination (skin and mucous membranes, lymph nodes, head, neck, chest, abdomen, spine/limbs), novel Coronavirus (SARS-COV-2) nucleic acid test and antibody test, urine pregnancy (women of childbearing age) test, and vital signs (blood pressure, axillary/oral temperature, pulse) evaluation.

Screening eligible subjects will be 1:1 randomly assigned to the experimental group and the placebo control group, and vaccinated as per the 0, 1, 2 month immunization schedule.

Safety evaluation:

AEs and SAEs:

All adverse events (AEs) up to 30 minutes after each dose of vaccination, all AEs from 0 to 7 days (including both solicited and unsolicited), and all AES from 8 to 30 days (unsolicited) will be collected;

All serious adverse events (SAEs) will be collected from the first dose of vaccination to 12 months after the whole vaccination.

Solicited AEs (the following events occurred within 7 days after

	vaccination): Injection site (local) adverse events: pain, swelling, induration, redness, rash, pruritus Vital Signs: fever Non injection site (systemic) adverse events: headache, fatigue / asthenia, nausea, vomiting, diarrhea, muscle pain (non-injection site), cough, acute allergic reaction, mental disorder (specific symptoms) **ADE/VED (Antibody Dependent Enhancement / Vaccine Enhanced Diseases) risk monitoring:** After vaccination (at least one dose of Investigational product), the subjects shall visit the hospital for hospitalization or isolation according to the local epidemic prevention and control requirements in case of confirmed COVID-19. Special investigation is needed for severe, critical or fatal cases, the DSMB shall analyze based on the results of special investigation whether ADE/VED phenomenon exists. **Efficacy evaluation:** The incidence rate and efficacy of any severity of COVID-9 of any severity 7 days after whole vaccination. **Immunogenicity and immune persistence evaluation:** Blood samples (5ml) will be collected before the first dose of vaccination and 14 days and 6 months after the whole course of vaccination to detect neutralizing antibody of SARS-COV-2 and protein binding antibody IgG of receptor binding region (RBD) .
Inclusion criteria	1) Population aged 18 years and above; 2) Subjects voluntarily participate in the study and sign the informed consent form; and are able to provide valid identification, and understand and comply with the requirements of the trial protocol; 3) Female subjects of childbearing age agree to use effective contraceptive measures from the beginning of the study to 2

	months after full course of vaccination.
Exclusion criteria	(1) Suspected or confirmed as fever(axillary temperature ⩾37.3° C / oral temperature ⩾37.5℃) within 72 hours before the enrollment, or axillary temperature ⩾ 37.3 ° C / oral temperature ⩾37.5℃ at the day of screening; (2) Diastolic blood pressure ⩾ 100mmhg and / or systolic blood pressure ⩾ 150mmhg; (3) Patients with previous history of a COVID-19; (4) Detection of SARS-COV-2 nucleic acid or antibody is positive; (5) Those who are suffering from the following diseases: a) With thrombocytopenia, any coagulation dysfunction or receiving anti-coagulatory treatment b) Congenital or acquired immune deficiency or autoimmune disease history; no spleen, or history of splenic surgery and trauma, or receiving immunomodulator treatment within 6 months, e.g., immunosuppressive dose of glucocorticoids (reference dose: equivalent to 20mg/ day of prednisone, over 1 week); Or monoclonal antibodies; Or thymosin; Or interferon etc.; However, topical application (such as ointment, eye drops, inhalers or nasal sprays) is permitted; c) Symptoms related to acute respiratory tract infection (such as sneezing, nasal congestion, runny nose, cough, sore throat, loss of taste, chills, shortness of breath, etc.); d) Cancer patients (except basal cell carcinoma) (6) With a history of serious allergy to any vaccine or any composition of Investigational product (including: aluminum preparations), such as allergic shock, allergic throat edema, allergic purpura, thrombocytopenic purpura, localized allergic necrosis reaction (Arthus reaction), dyspnea and angioneuroedema; (7) Inoculated with subunit vaccine and inactivated vaccine within 14 days before the first dosing of investigational? vaccine, or

	inoculated with attenuated live vaccine within 30 days; (8) Previous receiving blood transfusion or blood relevant products (including immunoglobulin) within 3 months, or planning to receive such products from the starting of study to <6 months after the whole-course inoculation; (9) Have participated in or are participating in other covid-19 related clinical trials; (10) Women in breastfeeding period or in pregnant period (including women at childbearing age with positive result of urine pregnancy test); (11) Considered by investigators as any disease or state possibly making the subject at unacceptable risk; not conforming to the requirements of study protocol; interference of assessment of reactions of vaccine.
Criteria for withdrawal	Withdrawal decided by the investigators: 1) AEs or concomitant conditions that discontinue the trial; 2) Having participated in other clinical trials before the end of this clinical trial; 3) Subjects with other conditions that are not suitable to participate in the clinical study, as considered by the investigator; Withdrawal requested by the subjects: subjects can freely discontinue the study participation at any time during the study.
Study Endpoints	**Primary endpoints:** (1) The endpoint of efficacy study: The number of any severity of COVID-9 cases 7 days after whole vaccination. (2) The endpoint of safety study: a. Analysis of adverse events from the first dose of vaccination until 30 days after full course of vaccination: incidence of adverse reactions or adverse events; incidence of grade 3 or above adverse reactions or adverse events; incidence of

	adverse reactions or adverse events leading to withdrawal. b. Analysis of serious adverse events from the first dose of vaccination until 12 months after full course of vaccination: incidence of serious adverse events; incidence of serious adverse events associated with Investigational product. **Secondary endpoints:** (1) The endpoint of efficacy study: a. The number of severe and severity above COVID-19 cases 7 days after whole vaccination; b. The number of any severity of COVID-9 cases after first dose of vaccination; c. The number of COVID-19 cases of any severity in populations of different age group (18-59 years vs. 60 years and above) 7 days after whole vaccination. (2) Endpoint of immunogenicity and immune persistence study: The level of neutralizing antibody to SARS-COV-2 and IgG level of RBD protein binding antibody at 14 days and 6 months after full course of vaccination. **Exploratory endpoint:** The protective level of neutralizing antibody to SARS-COV-2 and IgG of RBD protein binding antibody against COVID-19 caused by SARS-COV-2 infection.
Research Hypothesis	**Primary study hypothesis:** At least 7 days after full course of vaccination, the vaccine provide better protection against any severity of COVID-9 than the placebo (The lower bound of the 95% confidence interval is >30%).
Sample Size Considerations	**Sample size calculation based on efficacy study** A large-scale, confirmatory clinical study is needed to evaluate the protective effect of the vaccine on COVID-19 in people aged 18 years and above. The power is calculated based on the assumption of

	the incidence rate of any severity of COVID-9 during the trial to be 1%. An interim analysis is planned when 1/3 or 2/3 of the any severity of COVID-19 cases are observed, and the overall type I errors will be controlled within 5% (both sided) by using O'Brien - Fleming spending function. In order to test that the vaccine protection effect is no less than 60% (95%CI lower bound > 30%), 156 cases of any severity of COVID-9 and 22,286 subjects (11,143 subjects in each group) in total are required to achieve 90% power. The number of events is calculated by using the exact condition method under the large sample poisson distribution hypothesis of Chan and Bohidar. Therefore, taking into account the dropout, protocol deviation, non-compliance, 14,000 subjects are planned to be recruited in each group. **Sample size calculation based on immunogenicity bridging study** Set a non-inferior effect value of 0.67, the inspection level α of 0.025 unilateral, and a master degree of 90%, and assume that the GMT of anti-SARS-COV-2 neutralizing antibody in people in China and outside China is the same, the standard deviation of the antibody titer after logarithmic conversion is 0.55, and the distribution ratio of the two groups of samples is 1:1. Using PASS 15, the minimum sample size of each Investigational product group in China and outside China was 207. Considering factors such as shedding and age distribution, 1000 subjects were planned to be enrolled in each experimental vaccine group in China and outside China (750 subjects aged 18-59 and 250 subjects aged 60 and above).Therefore, a total of 20,000 patients are to be enrolled, including 1,000 in China and 1,000 outside China (1,000 subject outside China will be immunogenicity subgroup of the efficacy study cohort, which will also participate in the efficacy evaluation). There will be 1000 in the investigational vaccine group and 1000 in the placebo group. To sum up, the total sample size of the Phase III clinical trial will be 29,000. Because of the unpredictable number of cases in some countries and

<table>
<tr><td></td><td>centers and the change in incidence rate in countries, the number of COVID-19 cases will be reviewed in blinded manner during the trial. The number of subjects planned to be enrolled could be increased during the trial according to the change in incidence rate of different regions, but the number of any severity of COVID-9 cases required could not be changed, therefore, it will not cause inflation of the overall type I errors.</td></tr>
<tr><td>Suspension or termination criteria for the trial</td><td>In case that new data on the Investigational product in this study or any other study is available, and the regulatory authorities, sponsor, investigator, and/or institutional review board / independent ethics committee believe that the trial shoud be suspended/terminated;
Suspension criteria for the trial
In case of any of the following situations, the trial shall be suspended, and the institutional review board / independent ethics committee and relevant regulatory authorities shall be notified immediately, data safety monitoring board (DSMB) expert meeting will be held urgently for safety demonstration, analysis and determination on whether to continue this study.
<table>
<tr><th>Events leading to trial suspension</th><th>Number, %</th></tr>
<tr><td>Vaccine-related deaths or serious life-threatening adverse reactions occurred during the study period</td><td>⩾1 case</td></tr>
<tr><td>Grade 3 or above AEs that last 48 hours after any dose of vaccination</td><td>>15% of all vaccinated</td></tr>
</table>
Termination criteria for the trial
In case of any of the following situations, the trial shall be terminated, and the institutional review board / independent ethics committee and relevant
regulatory authorities shall be notified immediately.
<table>
<tr><th>Events leading to trial termination</th><th>Number/%</th></tr>
<tr><td>Grade 3 or above AEs that last 48 hours after any dose of vaccination</td><td>>30% of all vaccinated</td></tr>
</table></td></tr>
<tr><td>Data and safety</td><td>Safety data monitoring:</td></tr>
</table>

monitoring board (DSMB) Responsibility	A DSMB will be set up to monitor the safety data during the study. (1) Verify, approve and complete relevant work in time according to the stipulations in chapters of DSMB; (2) Verify study protocol, verify efficacy/safety data and raise suggestions for revision of monitoring plan; (3) Execute the verification of data at unblind state according to the monitoring plan. The efficacy/safety data are exhibited at unblind state through the information of actual study grouping (including: true name of two groups). (4) If the conditions are allowed, the factors beyond study are explored, such as scientific or therapeutic progress possibly causing a problem in safety of subjects or ethics of study. (5) Participate in discussion of DSMB, and vote for suggestions of DSMB when necessary; (6) Suggest the sponsor for other modifications during the course of study and after the termination of study based on the observed data; (7) If severe, critical or fatal case occurr after the subject is infected with SARS-COV-2 during study period, a special investigation should be conducted. The DSMB shall conduct an analysis based on the findings of the specific investigation. If the analysis suggests that ADE/VED exists, the DSMB shall convene an emergency meeting to assess the risk of ADE/VED in the entire trial and immediately report it to the institutional review board (IRB) / independent ethics committee (IEC) and relevant regulatory authorities.
Endpoint adjudication committee (EAC) responsibility	1) Able to review, approve and complete related work in a timely manner in accordance with the EAC charter; 2) The chairman is responsible for supervising whether the review of the endpoint event is carried out in accordance with the trial protocol; shall attend all meetings; record (only all the review results) in the summary report form and sign; responsible for checking meeting minutes and signing; coordinate and reach a

	consensus, and communicate the views to the sponsor; 3) In case of any endpoint event, objectively evaluate the endpoint event according to the unified definition standards, combined with clinical expertise and relevant contents in the protocol to determine whether it conforms to the definition standards; 4) Review the description of all events and check the source documents of each event. Necessary relevant information in the source documents obtained by the committee members have to be masked to ensure that blind review is achieved; 5) During the independent review process, the committee members can request to provide the required source documents, and review relevant clinical data (i.e., lung imaging, death certificate, hospitalization records, etc.) before making the final decision; 6) Members reach a consensus on the independent review, and the review results will be announced at the meeting and the chairman will sign for confirmation in the summary report form. If the independent review opinions of the committee members cannot reach a consensus, review meetings (regular meetings or ad hoc meetings) will be held as necessary to discuss them. If there are still disagreements after the discussion, voting shall be performed. Voting must also follow certain rules; 7) If the committee members need additional source documents during the review meeting, they should be recorded in the meeting minutes and make supplementary application after the meeting; 8) The EAC management team needs to cooperate with the data management department to complete the data question proposal and answer for the review results, which is different from the general clinical trial question answering process; 9) After completing evaluation, formulate the final Master Binder.
Case monitorin	After the first dose of vaccination, if the subjects have symptoms in consistent with suspected COVID-19, they must contact the

g	investigators in timely manner, and the investigators will determine the suspected case according to the suspected case definition. Subjects who are determined to be suspected cases shall visit the study facility or designated institutions for collection of throat swabs from the subjects, and undergo SARS-COV-2 nucleic acid detection by real-time fluorescent quantitative RT-PCR.
Definitions	1) "Month" in the visit: defined as "30 days"; 2) Women of childbearing age: refers to women in a specific period from the development of female reproductive organs (menarche) to ovarian function decline (menopause); 3) 18-59 years of age: 18 years old or above on the day of enrollment (i.e. the day of 18 years old), but less than 60 years old (i.e. the day before 60 years old); 4) 60 years and above: at least 60 years old on the day of enrollment (i.e., 60 years old) or above. 5) Suspected COVID-19: Those who meet any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia/nausea/vomiting, loss of smell/taste . 6) COVID-19: Suspected cases were sampled twice (two samples were collected for each time, one for testing and one for backup storage) , at a sampling interval of 24-48h. The result of Real-time fluorescence quantitative RT-PCR test for SARS-COV-2 nucleic acids was positive at least once and having any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia/nausea/vomiting, loss of smell/taste. 7) Mild COVID-19: Symptomatic patients with confirmed COVID-19 and no evidence of viral pneumonia or hypoxia, Having symptoms include fever, cough, fatigue, anorexia, shortness of breath, myalgia, sore throat, stuffiness, headache, diarrhea,

	nausea, vomiting, loss of smell (anosmia), loss of taste (ageus). 8) Moderate COVID-19: Confirmed COVID-19, having clinical signs of pneumonia (fever, cough, dyspnea, shortness of breath) but no signs of serious pneumonia, including SpO2≥ 90% under indoor air conditions. 9) Severe or critical COVID-19: including a) and b) a) Severe COVID-19: Confirmed COVID-19 and meet any of the following conditions:1) shortness of breath, RR ≥ 30 times / min; 2) under resting state and indoor air condition, SpO2＜90%; 3) arterial partial pressure of oxygen (PaO2) / oxygen inhalation concentration (FiO2) ≤ 300 mmHg (1mmhg = 0.133kpa); PaO2 / FiO2 should be corrected according to the following formula: PaO2/FiO2 x [760/atmospheric pressure (mmHg)]; 4) the clinical symptoms worsened progressively, and the lung imaging showed that the lesions progressed more than 50% within 24 to 48 hours. b) Critical COVID-19: Confirmed COVID-19, one of the following occurs， respiratory failure (high-throughput oxygen therapy, non-invasive ventilation, mechanical ventilation, ECMO), shock, admission to ICU, sepsis, complicated with acute pulmonary embolism, complicated with acute coronary syndrome, complicated with acute stroke, complicated with delirium, complicated with other organ failure, death, etc.
Duration of Study	Each subject will participate in the clinical trial for about 14 months.

2. Glossary of terms

Standard operating procedures	Standard and detailed written procedures to effectively implement and complete each task in one clinical trial.
Case report form	Paper or electronic document recording subject's relevant data reported to the sponsor that is designed as required in the study protocol.
Auditing	Systematic and independent inspection of clinical trial related activities and documents as to evaluate the implementation of clinical trial related activities, if the record, analysis and reporting of study data meet the study protocol, standard operating procedures and requirements in relevant laws and regulations.
Monitoring	One action to monitor the progress of clinical trial, and make sure the clinical trial is conducted, recorded and reported in accordance with the study protocol, standard operating procedures and requirements in relevant laws and regulations.
Investigational product	Vaccines for clinical trials, including investigational vaccine and placebo.
Randomization	The randomization principle in clinical trials is defined as the implementation process or measure in which each subject in the clinical trial has equal chance to be assigned to the study group or placebo group, the randomization process will not be affected by investigator's and/or subject's subjective will.
Blinding	Blinding is one of the important measures to control the bias resulted from awareness of randomized grouping information in clinical trials, and for the purpose of achieving the unpredictability of randomization by all the parties in clinical trials.
Subjects	Recipients of investigational vaccine who participate in one clinical trial, including patient, healthy subject.

Dropout	Defined as inability to receive the last follow-up required in the study protocol for any reason.
Investigator's Brochure	Clinical and non-clinical study data on the Investigational product available when conducting human trials.
Preventive drugs	The drugs for prevention of possible solicited AEs in the solicitation period after vaccination.

Informed consent form	Defined as the documentary evidence on each subject's willingness to participate in one trial.The property, objective, possible benefits and risks, the available other therapeutic options, rights and obligations for subjects in compliance with the Declaration of Helsinki need to be described by investigators to the subjects, allowing subjects to express their consent upon full awareness.
Adverse events	All the untoward medical events occurred in a subject after administration of an investigational vaccine, which do not necessarily have a causal relationship with the treatment.
Solicited adverse events	Adverse events collected as safety endpoints in the clinical study, defined as the data on the adverse events actively solicited by investigators or subjects within specific follow-up period after vaccination.
Unsolicited adverse events	Other adverse events except the solicited adverse events reported in clinical studies, also including the solicited adverse events reported beyond the designated time window of solicitation.
Serious adverse events	Death, being life-threatening, permanent or serious disability or loss of function, requiring hospitalization or prolonged hospital stay, as well as congenital anomaly or birth defects and other medical events after administration of Investigational product.
Data and safety monitoring committee	One independent committee comprised of professionals with relevant professional knowledge and experience, which is established by the sponsor for regular evaluation of the progress of clinical trial, safety data and key efficacy variables, and can give advice to the sponsor on continuation, modification or discontinuation of the trial.
Endpoint adjudication committee	One committee comprised of clinical experts who adjudicate the primary evaluation endpoints of the clinical trial according to standard working procedures. Regardless of the use of blinding in the clinical trial, the committee experts should be kept blind

	to the evaluated subject at the evaluation of endpoint.

3. Abbreviations

COVID-19	Coronavirus Disease 2019, a disease caused by SARS-COV-2 virus
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
EAC	Endpoint Adjudication Committee
RBD	Receptor Binding Domain
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
PI	Principal Investigator
IA	Interim Analysis
AE	Adverse Event
SAE	Serious Adverse Event
ADE	Antibody Dependent Enhancement
VED	Vaccine Enhanced Diseases
Arthus	Local Anaphylactic Necrosis Reaction
CI	Confidence Interval
IRB	Institutional Review Board
NMPA	National Medical Products Administration
SpO_2	Blood Oxygen Saturation
WHO	World Health Organization
SARS-COV	Severe Acute Respiratory Syndrome Coronavirus
SARS-COV-	Severe Acute Respiratory Syndrome Coronavirus type 2, the virus

2	that causes COVID-19
SOP	Standard Operation Procedure
MCHC	Mean Corpuscular Hemoglobin Concentration
MPV	Mean Platelet Volume
RDW	Red Cell Distribution Width
#NEUT	Neutrophil Count
%NEUT	Neutrophil Percentage
#LYMPH	Lymphocyte Count
%LYMPH	Lymphocyte Percentage
#MONO	Monocyte Count
%MONO	Monocyte Percentage
#EOS	Eosinophil Count
%EOS	Eosinophil Percentage
#BASO	Basophil Count
%BASO	Basophil Percentage
#LUC	Large Unstained Cell Count
%LUC	Large Unstained Cell Percentage
#RETIC	Reticulocyte Count
%RETIC	Reticulocyte Percentage
PT	Prothrombin Time
APTT	Activated Partial Thrombin Time
Fbg	Fibrinogen
RBC	Red Blood Cell Count
WBC	White Blood Cell Count
HGB	Hemoglobin
PLT	Platelet Count

HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
PBLC	Peripheral Blood Lymphocytes
IL-4	Interleukin-4
IL-2	Interleukin-2
IFN-γ	Interferon-gamma
CTL	Cytotoxic T Lymphocyte
NOAEL	No Observed Adverse Effect Level
GMT	Geometric Mean Titer
RT-PCR	Reverse Transcription Polymerase Chain Reaction
MERSR-COV	Middle East Respiratory Syndrome Coronavirus
VERO E6	African Green Monkey Kidney Cell Line
HUH-7	Human Hepatoma Cells
BAT-SL-COVZC45	Bat SARS-Like Coronavirus
NCP-RBD	Novel Coronavirus Spike Protein - Receptor Binding Region
ELISpot	Enzyme Linked Immunospot Assay
GMP	Good Manufacturing Practices
RR	Respiratory Rate
ECMO	Extracorporeal Membrane Oxygenation
CHO Cell	Chinese Hamster Ovary Cell
eCRF	Electronic Case Report Form
PV	Pharmacovigilance

4. Background and Rationale

4.1. Disease and pathogen

4.1.1. Disease setting

Since December 2019, there have been many cases of pneumonia patients infected by the Novel Coronavirus in Wuhan, Hubei Province. With the spread of the epidemic, such diseases have also occurred in other regions of China and abroad. As of October 21st, 2020, the total number of confirmed cases worldwidehas reached 41 million, the total number of deaths worldwide is more than 1.12 million. On January 31, 2020, the World Health Organization declared the outbreak of the Novel Coronavirus as a global public health emergency. On February 11, 2020, the World Health Organization announced that the Novel Coronavirus-infected pneumonia official name"COVID-19". Subsequently, the World Virus Classification Committee named the virus as severe acute respiratory syndrome Coronavirus type 2 (SARS-COV-2).

At present, the source of infection with the Novel Coronavirus is mainly patients infected by the Novel Coronavirus, and asymptomatic infected patients may also become the source of infection. The main transmission routes are droplet transmission, contact transmission, fecal-oral transmission, and respiratory aerosol transmission of different sizes. Based on current epidemiological investigations, the incubation period of the disease is 1 to 14 days, mostly 3 to 7 days. The main symptoms of patients include fever, fatigue, and dry cough. A few patients are accompanied by nasal congestion, runny nose, sore throat and diarrhea. Severe patients often develop dyspnea and/or hypoxemia one week after the onset. In severe cases, the symptoms rapidly progress to acute respiratory distress syndrome, septic shock, difficult to correct metabolic acidosis, and coagulopathy. Judging from the status of the currently admitted cases, most patients have a good prognosis, and a few patients are in critical condition. The prognosis of the elderly and those with chronic underlying diseases is poor. Symptoms in children are relatively mild.[1]

4.1.2. Pathogen background

The Coronavirus particle diameter is between 70-120 nm and contains a single non-segmented RNA genome with a length of 26-32KB, encoding and duplicating enzyme protein, spikes protein (S protein), envelop small membrane protein (E protein), membrane protein (M protein) and nucleocapsid protein (N protein)[2] from 5 prime end to 3 prime end. Among these structural proteins, S protein mediates the

adhesion and entry of Coronavirus into host cells. The receptor-binding domain (RBD) is low in conservation among different viruses, and contains a variety of conformational neutralization tables. This allows it to easily spread across a wide range of hosts across cell and tissue types and even species barriers. The diversity of RBD also determines the types of host receptors used by different Coronaviruses and the way they enter host cells. SARS-COV binds to angiotensin-converting enzyme 2 (ACE2) receptors, and MERS-COV binds to two Dipeptidyl peptidase 4 (DPP4) receptor. The latest research shows that SARS-COV-2 may also enter human cells by binding to the ACE2 receptor. The above structural features provide a structural basis and theoretical basis for designing specific vaccines for different Coronaviruses.[2][3][4-5][6]

The Novel Coronavirus belongs to the beta genus of Coronaviruses. It has an envelope, and the particles are round or oval, often pleomorphic, with a diameter of 60-140nm. Its genetic characteristics are significantly different from SARS-COV (Severe Acute Respiratory Syndrome Coronavirus) and MERS-COV (Middle East Respiratory Syndrome Coronavirus). Current research shows that the homology with bat SARS-like Coronavirus (bat-SL-CoVZC45) is more than 85%. When isolated and cultured in vitro, SARS-COV-2 (Novel Coronavirus) can be found in human respiratory epithelial cells in about 96 hours, while it takes about 6 days in Vero E6 (African green monkey kidney cell line) and Huh-7 cells (human liver cancer cells). Most of the understanding of the physical and chemical properties of Coronavirus comes from the study of SARS-COV and MERS-COV. Viruses are sensitive to ultraviolet rays and heat. At 56°C for 30 minutes, 75% ethanol, chlorine-containing disinfectants, peracetic acid and chloroform and other lipid solvents can effectively inactivate the virus, but chlorhexidine cannot effectively inactivate the virus.[1]

4.2. Investigational product

4.2.1. Product characteristics

This vaccine is made by purified receptor binding region of the Novel Coronavirus spike glycoprotein (recombinant protein NCP-RBD) expressed by recombinant CHO cells and adding aluminum hydroxide adjuvant. It is used to prevent respiratory diseases caused by Novel Coronavirus infection. It is a milky white suspension liquid, which can be stratified due to precipitation and after shaking, it should be easy to disperse.

4.2.2. Product specification

The company's quality standards for the Recombinant Novel Coronavirus Vaccine (CHO cells) was formulated according to the research data of quality and registration standards, along with the *"Regulations for the Administration of Drug Registration"*, the *"Registration Classification and Application Data Project Requirements for Biological Products"*, ICH guidelines, and *"Technical Guidelines for Quality Control of Human Recombinant DNA Products"* , *"Chinese Pharmacopoeia"* (2015 edition), see the table below.

Table 4 Quality Standards of Recombinant Novel Coronavirus Vaccine (CHO Cell)

No	Test items	Quality control standards
1	Identification test	Coronavirus NCP-RBD antigen protein should be detected by enzyme-linked immunosorbent assay (ELISA).
2	Appearance	It should be a milky white suspension liquid, which can be stratified due to precipitation. After shaking, it should be easy to disperse, and there should be no lumps.
3	Quantity	According to law (General Rule 0102), it should not be less than the marked quantity.
4	Osmolality	280mOsmol/kg±65mOsmol/kg（General Rule 0632）
5	pH	5.0~7.0（General Rule 0631）
6	Aluminum content	0.35~0.65mg/ml（General Rule 3106）
7	Efficacy test	Vaccinate 10 female Balb/c mice of 4 to 8 weeks of age with test samples of each specification. Each mouse is injected intraperitoneally with 0.5ml test product. The vaccine reference product is used as a parallel control, and physiological saline is used as a negative control. After 2 weeks, blood is collected, and the level of anti-NCP-RBD antibody is detected by enzyme-linked immunosorbent assay, and the GMT value is calculated. The GMT ratio of the test product/vaccine reference product with a specification of 50μg/dose should be no less than 0.4, and the GMT ratio of the test product with a specification of 25μg/dose should be no less than 0.4. The GMT ratio of the test product/vaccine reference product should be no less than 0.2.
8	Sterility test	According to the rule（General Rule 1101），should be in compliance..
9	Abnormal toxicity test	According to the rule（General Rule 1141），should be in compliance..
10	Bacterial endotoxin test	Should be less than 10EU/ml（General Rule 1143 Gel

No	Test items	Quality control standards
		limit test method）

4.2.3. Stability studies

The company's plan for studying the stability of the Recombinant Novel Coronavirus Vaccine (CHO cell) bulk was formulated according to the *"New Drug Approval Regulations* ", "*Chinese Pharmacopoeia"* (2015 Edition) and the *"Technical Guidelines for Stability Research of Biological Products".*

The research of the stability of the bulk solution mainly includes: long-term stability study (2~8℃/-20±2℃ storage for 18 months), accelerated stability test (25 ±2℃) and mandatory conditions (repeated freezing and thawing, high temperature, vibration) test. During the entire inspection process, samples were taken at each time point, and tested according to different test items. The basic data can be used for reference in production and storage during scale-up production. The samples investigated in this study are shown in the table below.

Table 5 Conditions, research indicators and research results of vaccine stability studies

Types of tests		Environmental conditions	Planned sampling time point	Test items
Mandatory condition test	Shaking test	2~8℃, shaking	7、14、21、28 day	On the 7th, 14th and 21st day, test appearance, pH value, bacterial endotoxin and efficacy.
	Illumination test	2~8℃, Light intensity 4500lx±500lx	7、14、21、28 day	
	High temperature test	37±2℃	7、14、21、28 day	
Accelerated stability		25±2℃	1、2、3、6 month	Test appearance, pH, bacterial endotoxin and efficacy in the 1st month, test appearance, pH, bacterial endotoxin in the 2nd and 3rd month, test appearance, pH, bacterial endotoxin, sterility inspection, and efficacy in the 6th month.

Long-term stability	2~8℃	0、3、6、9、12、18、24、30 month	Only test the appearance, bacterial endotoxin and pH value in the 3rd, 9thand 18thmonth, and full inspections will be conducted at 0, 6th, 12th, 24th and 30th month.

At present, the research of mandatory condition stability test, accelerated stability test and long-term stability test of the vaccine is still in progress. All tests arecompleted and within the qualified rangeStability tests under mandatory conditions of the high temperature, shaking, and illumination have been monitored for 28 days, and the appearance, pH, bacterial endotoxin and efficacy tests have been completed in compliance with the regulations.

The accelerated stability testhas been completed observation for 6 months The appearance, pH, efficacy and bacterial endotoxin tests have been completed, all the samples are in compliance with regulations. The long term stability test also has been completed the observation for 6 months, and all results of the tests are in compliance with regulations.

In conclusion, the observation results so far show that the vaccine has good stability, and can be stored at 2 ~ 8 ℃ for at least 30 months

4.2.4. Storage conditions

The vaccine should be stored under the temperature of 2 ~ 8 ℃, away from light and strictly prevent freezing. Temperature should be monitored and recorded daily during storage.

4.3. Nonclinical studies of the vaccine

4.3.1. Pharmacology studies

4.3.1.1. Primary pharmacology

4.3.1.1.1. Immunogenicity analysis in mice

(1) Binding antibody level result

In this study, Balb/c mice were immunized with this vaccine (immunization schedule is 0D-14D). 14 days after the second immunization, blood was collected and sera was separated for detection of NCP-RBD antigen-specific Binding antibody levels.The GMT value of antibody titer can reach as high as $10^{5.}$

The results showed that the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of high level of antigen-specific Binding antibody titer in mice after immunizing, indicating that the vaccine has good immunogenicity.

(2) Live virus neutralizing antibody level detection

The sera of the mice immunized with two injections of the above vaccine were tested by the Institute of Microbiology, Chinese Academy of Sciences for the level of live virus neutralizing antibodies. The GMT value of neutralizing antibody titer was 228.1

The results showed that after immunization in mice, a strong neutralizing affect is detected by authentic virus neutralizing antibody test, which proves that the vaccine has good immunogenicity.

4.3.1.1.2. Immunogenicity analysis in rats

In this study, rats were immunized intramuscularly with the vaccine and the cellular and humoral immune responses in rats were studied. The SD rats, SPF grade, 120 in total, half male and half female (age before administration: 7-9 weeks old, weight before administration: male 260-330g, female 179-234g) were divided into 4 groups by weight balancing randomized grouping method. There were 30 animals in each group, which were the blank control group, adjuvant control group, low-dose group and high-dose group. The four groups were given intramuscularly with 0.5 mL of 0.9% sodium chloride injection/ animal, placebo (including aluminum adjuvant) 0.5mL/animal, 0.25 mL vaccine (1/2 human dose)/animal, 0.5mL vaccine (1 human dose)/animal respectively. The animals were administered 3 times on D1, D15, and D29, and after full course of administration, the animals were observed for 2 weeks (recovery period).

(1) Binding antibody level result

The rat immunogenicity study of the Recombinant Novel Coronavirus Vaccine (CHO cells) was carried out along with repeated dose toxicity test in rats for 4 weeks. After three doses of immunization, blood was collected for GMT (NCP-RBD) testing during the withdrawal and recovery period.

The results showed that the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of antigen-specific IgG antibodies in the SD rats after immunization. After the third immunization, there was no significant difference in GMT (NCP-RBD) between the high and low dose groups during the withdrawal examination and recovery period, and there was no significant difference in GMT (NCP-RBD) between the different dose groups during the withdrawal and recovery

period. However, the GMT (NCP-RBD) value of binding antibody level in both the high and low dose groups reached 10^6, indicating that the vaccine could induce the production of high titer Binding antibody after immunization in rats, showing good immunogenicity.

(2) Pseudovirus neutralizing antibody test result

The rat immunogenicity study of Recombinant Novel Coronavirus Vaccine (CHO cell) was carried out with repeated administration toxicity test in rats for 4 weeks. After third immunizations, the sera of female rats during the withdrawal period was tested for the level of pseudovirus neutralizing antibodies.

The results showed that this vaccine can induce the secretion of antigen-specific Binding antibody and pseudovirus-neutralizing antibody in rats (SD). After the third immunization, Binding antibody the antibody titer of pseudovirus-neutralizing antibody can reach 10^3, which verifies the good immunogenicity of this vaccine.

4.3.1.1.3. Immunogenicity analysis in crab-eating macaques

This study was carried out along with repeated dose toxicity test in monkeys for 10 weeks (0-4w-8w-10w). Crab-eating macaques were immunized intramuscularly to study the effect of the vaccine in inducing cellular and humoral immune responses in primates. 40 general-grade crab-eating macaques qualified for quarantine were selected, half male and half female (age before administration: 3 to 4 years old, male weight 2.95 to 3.67 kg, female weight 2.97 to 3.62 kg) and randomly divided into 4 groups, namely blank control group, adjuvant control group, low-dose group, high-dose group, with 10 animals in each group (♀♂ half each). The blank control group was injected intramuscularly with 0.9% sodium chloride injection 1.0 mL/animal, and the adjuvant control group was injected intramuscularly with 1.0 mL vaccine placebo (containing aluminum adjuvant) per each animal. The low and high dose groups were injected intramuscularly 0.5mL (1 human dose)/animal and 1.0mL (2 human doses)/animal, for a total of 4 administrations (at week 0, week 4, week 8, week 10), after full course of administration, the animals were observed for 2 weeks (recovery period).

(1) Binding antibody level result

Crab-eating macaques were immunized with the vaccine according to the procedure of week 0-week 4-week 8-week 10. Blood was collected before the first

dose, before the second dose, before the third dose, before the last dose and at the end of the recovery period.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of antigen-specific IgG antibodies in Crab-eating macaques. As the number of immunization times increases, the corresponding GMT increases accordingly. After the second immunization, the antibody level can reach the level of 10^7. It shows that the vaccine has good immunogenicity on crab-eating macaques; there is no significant difference in the level of antibodies produced by the high-dose group and the low-dose group during different immunization doses, indicating that different immunization dosage have no significant impact on the production of antibodies; For both the high-dose group and the low-dose group, there was a significant difference between the second dose and the third dose, and there was no significant difference between the antibody levels before the third dose, before the last dose, and during the recovery period. Therefore, the number of immunizations for this vaccine should be no less than two doses.

(2) Pseudovirus neutralizing antibody test result

In this study, blood samples of the crab-eating macaques were collected before the first immunization, before the second immunization, before the third immunization, before the last immunization, and during the recovery period to test the levels of pseudovirus neutralizing antibodies.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of neutralizing antibodies in crab-eating macaques. As the number of immunization times increases, the corresponding pseudovirus neutralizing antibodies increase accordingly. After the second immunization, the antibody level can reach 10^4, indicating that the vaccine humoral immunity induced by the vaccine in crab-eating monkeys have potential high neutralization effect on virus;; for the high-dose group, there is a certain difference before the third immunization and before the last immunization, but there is no significant difference between the third immunization and the recovery period; In the low-dose group, there was a significant difference before the third immunization and before the last immunization, and there was no significant difference before the last immunization and the recovery period; for the high-dose group and the low-dose group, before the third dose, there was a significant difference in the levels of pseudovirus neutralizing antibodies (*$P<0.05$),

and there was no significant difference in the levels of pseudovirus neutralizing antibodies before the last administration and during the recovery period.

(3) Live virus neutralizing antibody test result

In this study, blood sample of the crab-eating macaques were collected before the third immunization and before the last immunization, and the level of authentic virus neutralizing antibodies was tested.

The results showed the Recombinant Novel Coronavirus Vaccine (CHO cells) can induce the secretion of neutralizing antibodies against the authentic virus in crab-eating macaques, and after the second immunization, the level of neutralizing antibody reached 10^3, indicating that the humoral immunity induced by the vaccine in crab-eating monkeys can effectively neutralize the live virus. There was no significant difference in neutralizing antibody levels among the high-dose group and the low-dose group and there was no significant difference between the high-dose group and the low-dose group before the third dose and before the last dose. After the second immunization, the level of neutralizing antibody reached 10^3.Therefore, there is no significant difference in authentic virus neutralizing antibody level among crab-eating macaques administered with different dosages.

(4) Cellular immune test results

The IL-2 ELISPOT, IFN-γ ELISPOT and IL-4 ELISPOT tests were carried out during the drug withdrawal and recovery period in the 10-week repeated dose toxicity test of the Recombinant Novel Coronavirus Vaccine (CHO cells) in monkeys (i.m. injection). The results are as follows:

1) IL-2 ELISPOT test results: the spleen lymphocytes in both high and low dose groups produced effective and strong cellular responses against the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, *P<0.05), indicating that the antigen could effectively stimulate the secretion of IL-2 cytokines by spleen lymphocytes.

2) IFN-γ ELISPOT test results: The spleen lymphocytes in both high and low dose groups produced effective and strong cellular responses against the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, *P<0.05), indicating that the antigen could effectively stimulate the secretion of IFN- cytokines by spleen lymphocytes.

3) IL-4 ELISPOT test results: The spleen lymphocytes in both high and low dose groups produced effective and strong cellular responses to the antigen NCP-RBD, which were significantly different from those in adjuvant control group and blank control group (***P<0.001, **P<0.01), indicating that the antigen could stimulate the secretion of IL-4 cytokines by spleen lymphocytes.

In summary, the results of the antigen-specific IL-2 ELISpot, IFN-γ ELISpot, and IL-4 ELISpot experiments of Crab-eating macaques during the drug withdrawal period and the end of the recovery period show that after injection of the Recombinant Novel Coronavirus Vaccine (CHO cells) in crab-eating macaques, the spleen lymphocytes of the high and low dose groups had a strong cellular response to the NCP-RBD antigen which is of significant difference comparing to the adjuvant control group and the blank control group, indicating the vaccine has good cellular immune effectin primates.

4.3.1.1.4. Pharmacology study conclusions

In summary, similar to the results of studies in mice and rats, this vaccine can also efficiently induce humoral immune responses in crab-eating macaques. The detection and evaluation of antibodies in animal sera show that immunization of different animals with this vaccine can induce the body to produce high levels of neutralizing antibodies. The results of the antigen-specific IL-2 ELISpot, IFN-γ ELISpot, and IL-4 ELISpot experiments of the Crab-eating macaques monkeys during the drug withdrawal inspection period and the end of the recovery period show that the vaccine can effectively stimulate specific cellular immune responses in the Crab-eating macaques after immunization. All results indicate that the vaccine has good immunogenicity and can induce a wide range of humoral and cellular immune responses.

4.3.1.2. Challenge test

Animal protection research is an important part of evaluating the immune effect of vaccines. Through the study of Coronavirus challenge on the Recombinant Novel Coronavirus Vaccine (CHO cells) ACE2 transgenic mice, to evaluate the anti-infection effect of ACE2 transgenic mice after immunization. Mice were immunized with one injection on day 0 and day 21, 0.1ml/mouse (containing 10μg of antigen), and challenged 7 days after the two doses, the challenge dose was 5×10^5 TCID50/mouse.

Experiment conclusions: 1) After the control group was challenged, the body weight decreased significantly in the first 3 days. The virus titer of lung tissue reached $10^{5.32}$-$10^{7.99}$ TCID50 per gram. The lung lesions are obvious, with severe interstitial pneumonia. 2) After the immunization group was challenged, the weight loss was slight in the first 3 days. The virus titer of lung tissue reached 10^{2}-$10^{3.8}$ TCID50 per gram, a decrease of $10^{1.52}$-$10^{5.99}$ compared with the control group, and an average decrease of 3.6 Log times (3981 times). Lung lesions are mild, with mild interstitial pneumonia. It suggests that vaccine immunity has obvious protective effect.

4.3.1.3. Safety Pharmacology Studies

Crab-eating macaques were given 0.9% sodium chloride injection 1.0 mL/mouse (blank control group), Recombinant Novel Coronavirus Vaccine (CHO cells) placebo 1.0 mL/mouse (adjuvant control group), Recombinant Novel Coronavirus Vaccine (CHO cells) 0.5mL/mouse (low-dose group) and 1.0mL/mouse (high-dose group) have no significant effect on the ECG, blood pressure, respiration, body temperature and other indicators of Crab-eating macaques. The ECG, blood pressure, respiration, body temperature and other indicators of the adjuvant control group, low-dose group, and high-dose group were basically similar to those of the blank control group at the corresponding time ($P>0.05$). The fluctuations of blood pressure, respiration, body temperature and other indicators are caused by normal stress changes or circadian rhythms of monkeys, and have nothing to do with the test product.

The blank control group, adjuvant control group, low-dose group, and high-dose group showed consistent changes in the ECG, blood pressure, respiration, and body temperature indicators, that is, the change trend of each index is similar in the time period of -1h to 5h after administration, 5-6h after administration, and 6-24h after administration, and there was basically no difference among groups. The fluctuations of blood pressure, respiration, body temperature and other indicators are caused by normal stress changes or circadian rhythms of monkeys, and have nothing to do with the test product. It is comprehensively judged that the NOAEL of the Recombinant Novel Coronavirus Vaccine (CHO cells) on the cardiovascular and respiratory systems of Crab-eating macaques is greater than 1.0 mL (2 servings) per mouse.

Therefore, under the conditions of this test dose, crab-eating macaques were given a single intramuscular injection of the Recombinant Novel Coronavirus Vaccine (CHO cell) developed by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. with a batch

number of 202004003 to affect the cardiovascular and respiratory systems of Crab-eating macaques. There is no obvious effect, and the NOAEL of cardiovascular and respiratory system safety pharmacology is greater than 1.0mL (2 servings, 100μg NCP-RBD)/head.

4.3.2. Toxicology studies

4.3.2.1. Single-dose study

4.3.2.1.1. Single dose toxicity study in Sprague Dawley (SD) rats

Rat single-dose toxicity test: select SD rats, half of the male and female, 10/sex/group, intramuscular injection on hind limbs, single dose; maximum dose or maximum tolerated dose, set according to the results of the preliminary test. 3 groups: solvent control group, adjuvant control group, vaccine group (2 human dose). Clinical observation, body weight; gross anatomy 14 days after immunization (if abnormal, conduct pathological tissue examination), make pathological section of injection site.

Under different dose conditions of this test, rats were injected intramuscularly with Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) 2 human dose /animal and Recombinant Novel Coronavirus Vaccine (CHO cells) placebo (containing aluminum) (batch number: 202003002) (adjuvant reference substance) 2 human dose /animal, nodule at the injection site appeared after the immunization of the rat, and no abnormal reaction was seen in the test, nor did it cause the death of the rats; there was no significant effect on the weight gain of the rat.

Pathological examination results showed that all rats in the immunization group and the adjuvant control group had obvious inflammatory reactions, adjuvant deposition foci, and lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. The pathological changes in the two groups were basically consistent, considering the typical reaction at the site of immunization of aluminum-containing adjuvants given by injection, no abnormal lesions were seen in other tissues and organs, and no toxic target organs were clearly shown.

Based on the above test results, the maximum dose of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) for a single intramuscular injection in rats is 2 human dose /animal, and the maximum tolerable dose >2 human dose /animal

4.3.2.2. Repeated dose studies

4.3.2.2.1. 4-week repeated dose toxicity study in SD rats

120 adult (10~12w) SD rats, 15 rats/type/group were tested. Intramuscular injection 1 dose/week, 3 times (N+1), namely injection at 0W, 2W, 4W; 4 groups: Solvent control group, adjuvant control group, low-dose group (0.5 human dose), high-dose group (1 human dose).

Observation indicators are: (1) routine indicators: clinical observation: once a day; weight, food intake: once a week; clinical examination (hematology, blood coagulation, blood biochemistry, electrolyte, bone marrow smear), gross examination (Including the weighing of main organs) and a full set of histopathological examinations: stop medication and once at the end of the recovery period. (2) Immunological index inspection: lymphocyte immunophenotype analysis, sera immunoglobulin (IgG), complement (C3, C4), once vaccine withdrawal and recovery period; (3) Antibody test: At the end of the withdrawal period and recovery period, blood samples were collected once for detection of antibodies (IgG) and neutralizing antibodies titers.

Under the dosage conditions of this test, rats were given intramuscular injections of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) 0.25 mL (0.5 human dose) for 4 consecutive weeks (total immunization 3 times, 1 time/2 weeks) only, 0.5 mL (1 human dose) per animal, and Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (containing aluminum) (batch number: 202003002) 0.5 mL per animal, mainly as follows: adjuvant control group, high-dose group. After the immunization of the rats, the nodules can be touched at the injection site (without recovery after 2 weeks after stopping the vaccine), the rest has no obvious abnormal reaction and no animal death; no obvious abnormal effect on the weight gain and food intake of the rats; During drug inspection, it was found that there were no obvious abnormalities in the urine and ophthalmological indicators of each group; hematological indicators can be seen in all the three groups. MPV increased and PT decreased; biochemical indicators can be seen in all the three groups. Na^+ and Cl^- increased in the dose group, GLO increased and A/G decreased in the high-dose group.

In the withdrawal period, the immunotoxicity index examination showed that the low and high dose groups of IgG, C3 and the number of spleen systems of male rats

were correlated with increased doses, and the immune phenotype of peripheral blood lymphocytes was not abnormal; immunogenicity tests showed low and high dose groups vaccine can induce high-titer anti-NCP-RBD IgG antibodies with good immunogenicity in rats.

The results of histopathological examination showed that all rats in all the three groups showed obvious inflammatory reactions, adjuvant deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. The degree of change of the adjuvant control group is similar to the high-dose group, and the low-dose group is slightly less severe. Considering the typical reaction of intramuscular injection of aluminum-containing adjuvant at the injection site, there were no abnormal lesions in other tissues. The necropsy at the end of the recovery period generally showed that the local effects of the above-mentioned immunization showed a certain recovery trend.

In summary, rats have been injected intramuscularly (3 times in total) of Recombinant Novel Coronavirus Vaccine (CHO cells) for 4 consecutive weeks, 0.25 mL (0.5 human dose) per animal, 0.5 mL (1 human dose) per animal. It caused nodules at the injection site, some blood biochemical index effects (MPV immunization, PTV, Na^{+}↑, Cl^{-}↑), inflammatory reaction at the injection site, adjuvant deposition foci, and lymph nodes at the injection site similar to the adjuvant control group Hyperplasia and lymphatic sinus phagocytosis, the above abnormalities may be related to vaccination containing aluminum hydroxide adjuvant vaccine; it may also cause immune-related indicators (GLO drugs, A/G drugs, IgG drugs, C3G, spleen system number ↑). It is related to the immune response after vaccination. Excluding the above effects, no obvious toxic effects were seen during the drug withdrawal inspection stage, and the toxic target organs were not clearly displayed. The inspection results will be further analyzed after the recovery period.

4.3.2.2.2. 4-week repeated dose toxicity dose ranging study in monkeys

Choose crab-eating macaques, half of the male and female, 4/sex/group, 8 in total. Set up 2 groups: (1) adjuvant control group (2) vaccine group (1 human dose). Intramuscular injection on hind limbs, a total of 3 immunizations (N+1), namely 0w, 4w, and 8w, once each.

During the whole trial period, clinical observation (once a day); weight, food intake (once a week); ECG, body temperature, clinical tests (blood routine, blood

coagulation, blood biochemistry, ophthalmology, urine, etc.): immunization Before, after the second injection, 3D (interim immunization check), 3d after the last injection (drug discontinuation check), 1 time; urine check 1 time before autopsy. 3 days after the last injection (the end of administration), anatomy was performed according to routine requirements, and the main organs were weighed, bone marrow smear (smear preparation), gross and pathological examinations were performed.

This part of the toxicology study was commissioned by the Safety Evaluation Research Center of Zhejiang Academy of Medical Sciences. The monkey intramuscular injection of Recombinant Novel Coronavirus Vaccine (CHO cells) was repeated for 4 weeks and the toxicity test has been completed for 0w and 2w.

In summary, under the dosage conditions of this test, the Recombinant Novel Coronavirus Vaccine (CHO cell) with batch number 202004003 provided by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd. was subjected to 3 consecutive injections (D1, D15, D29). After injection, it has strong immunogenicity to crab-eating macaques, which can elicit strong cellular immune response and high titers of IgG antibodies; except for local effects of injection, no obvious toxic effects and toxic target organs are seen. No obvious systemic adverse reactions were observed at the dose of 0.5mL/animal (1 human dose).

4.3.2.2.3. 10-week repeated dose toxicity study in monkeys

Choose crab-eating macaques, ♀♂ half each, 5/sex/group, a total of 40. Set up 4 groups: (1) solvent control group (2) adjuvant control group (3) low-dose group (1 human dose) (4) high-dose group (2 human dose). Intramuscular injection on hind limbs, a total of 3 injection s (N+1), namely 0w, 4w, 8w, 10w once each. The recovery period is 2w.

During the entire test period, clinical observations were carried out every day (1 time a day during the adaptation period and recovery period, and 1 time each morning and afternoon during the administration period). After the injection, the local reaction of the animal administration site was observed (before each injection), observe once each at ~0.5h, ~1h, ~2h and ~6h after injection). Body weight and food intake are measured once a week; body temperature adaptation period is checked twice, each time before dosing, 4-6h after dosing, the next day and the third day of dosing. Electrocardiogram, ophthalmology, and clinical examination (blood routine, blood

coagulation, blood biochemistry) were checked twice during the adaptation period, and once each at the end of the drug withdrawal check and the recovery period; and once each of the urine routine withdrawal check and the end of the recovery period. PBLC immunophenotype, immunoglobulin (IgG, IgM), and complement (C3, C4) are checked once before administration, once drug withdrawal, and after the recovery period; specific anti-drug antibodies and neutralizing antibodies are checked each time before stopping the vaccine (3 days after the last dose) and at the end of the recovery period (15 days after the last dose), check once each. After the last injection 3d (drug withdrawal inspection), 15d (end of recovery period) according to the routine requirements, the main organs were weighed, bone marrow smear (smear preparation), gross and pathological examination, and spleen lymph was used cells were tested for CTL activity.

Crab-eating macaques were injected intramuscularly with the Recombinant Novel Coronavirus Vaccine (CHO cells) of batch number 202004003 for 10 weeks (4 times in total, 1 time each for 0w, 4w, 8w, and 10w) 0.5mL (1 human dose)/monkey, 1.0mL (2 human dose)/monkey, the Recombinant Novel Coronavirus Vaccine (CHO cell) placebo with batch number 202003002 (containing aluminum) 1.0mL/monkey, there is no obvious abnormal reaction in general clinical observation and local injection site, and no death ; No obvious abnormal changes in body weight, food intake, body temperature, blood biochemistry, electrocardiogram, urine, ophthalmology, organ weight and coefficient; drug withdrawal inspection, adjuvant control group, low-dose group and high-dose group %NEUT (Percentage of neutrophils) and #NEUT (number of neutrophils) increased, %LYMPH (percentage of lymphocytes) and #LYMPH (number of lymphocytes) decreased, and %EOS (percentage of eosinophils) and # in the high-dose group The increase in EOS (eosinophil count) reflects the appearance of typical inflammatory leukocyte blood picture after local intramuscular injection of the test product and adjuvant reference product containing aluminum adjuvant. The increase in EOS is also the performance of protein vaccine immunity, however, at the end of recovery period (2 weeks after drug withdrawal), the above abnormalities were down-regulated or up-regulated and returned to the reference range.

The results of histopathological examination showed that the pathological changes of the monkeys in the adjuvant control group and the low and high dose

groups were mainly at the injection site and its lymph nodes. Examination at the end of the recovery period showed that all monkeys had obvious inflammatory reactions and adjuvants at the injection site. Deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site. At the end of the recovery period, the above changes showed certain signs and trends for recovery; the above three groups of test monkeys were mostly consistent in the degree of lesions at the drug administration site. The high-dose group had lesions of relatively severe degree, the adjuvant control group and the low-dose group have relatively mild lesions. The main consideration is the typical reaction of intramuscular injection of aluminum-containing adjuvant at the administration site; no other tissues and organs were found in the two periods. Product-related abnormal pathological changes.

The immunogenicity results showed that both the low and high dose could produce strong humoral immune responses (producing high titers of antigen-specific IgG antibodies, pseudovirus and true virus neutralizing antibodies) and cellular immune responses (specific Sex antigen stimulates the secretion of IL-2, IFN-γ and IL-4 of splenic lymphocytes to increase significantly) in crab-eating macaques, and has good immunogenicity. This product has immunotoxicity indicators for immunoglobulin (IgA, IgG), complement (C3, C4), peripheral blood PBLC immunophenotype, immune-related organ weight and coefficient, macroscopic and histopathological indicators of immune-related organs No obvious impact was seen.

To sum up, crab-eating macaques were injected intramuscularly with 0.5 mL (1 human dose) of Recombinant Novel Coronavirus Vaccine (CHO cells) for 10 consecutive weeks (4 times in total, once each for 0w, 4w, 8w, and 10w). (2 human dose)/animal, it can mainly cause inflammatory reaction at the injection site and adjuvant deposition foci, lymph node hyperplasia and lymphatic sinus phagocytosis at the injection site similar to the adjuvant control group (there is a tendency to recover 15 days after stopping the drug), NEUT increased and LYMPH decreased. The above-mentioned abnormalities may be mainly related to vaccination of aluminum-containing adjuvant vaccines; it may also cause an increase in EOS, which may be related to the immune response of the vaccine. Excluding the above effects, under the dose conditions of this test, crab-eating macaques were injected intramuscularly with Recombinant Novel Coronavirus Vaccine (CHO cells) for 10 consecutive weeks (4 times in total). No obvious systemic and immunotoxicity effects were seen, and the

target organs of toxicity were not obvious. It showed that the NOAEL dose was 1.0 mL (2 human dose, NCP-RBD protein 100μg)/head.

4.3.2.3. Other toxicity studies

4.3.2.3.1. Local injection site reactions

Twelve New Zealand rabbits, were randomly divided into 3 groups, 4 rabbits in each group, half male and half female, and quadriceps femoris were injected with a single injection of Recombinant Novel Coronavirus Vaccine (CHO cells), adjuvant control and solvent control group (left and right) 0.5ml on each side), 1/2 animals in each group were anesthetized and executed 48 hours after administration, and the quadriceps femoris changes at the injection site were dissected to observe the changes in the quadriceps muscle at the injection site, scored according to the muscle stimulation response grading standard, and histopathological examination was performed. Leave 1/2 animals in each group to continue to observe until 14 days after the administration and then undergo histopathological examination to understand the reversibility of the irritation response.

According to the naked eye and histopathological examination, the local pathological changes in the quadriceps femoris muscle of one rabbit in the negative control group after 48 hours of drug withdrawal were considered to be caused by mechanical damage to the injection operation; the placebo group and the test product group are stopped For 48 hours, the experimental rabbits were examined for focal adjuvant deposition and acute inflammatory reaction at the injection site. At the end of the recovery period, the interstitial chronic inflammatory reaction and obvious adjuvant deposition at the injection site were examined. Both were administered with the injection of aluminum adjuvant. The typical local reactions are the same, so the above changes are considered to be the typical local reactions of intramuscular injection of aluminum-containing adjuvants.

In the placebo group and the test product group, the local changes of the experimental rabbits were mostly interstitial changes. The necrosis of muscle fibers on one side of the placebo group was checked for 48 hours after the drug was stopped. It may be due to individual differences or mechanical damage and adjuvant deposition during injection The reaction is caused by the combined effect, and the muscle fiber atrophy at the end of the recovery period is considered to be caused by the local deposition of adjuvant. In general, the substantial irritant damage of the muscle fibers

at the administration site of the placebo group and the test product group was not obvious, and was classified as "minor" according to the "Irritation Grade Judgment Standard".

Under the dosage conditions of this test, according to the average score of muscle stimulation response, according to the "Irritation Grade Judgment Standard", the Recombinant Novel Coronavirus Vaccine (CHO cell) (batch number: 202004003) showed a slight irritation response to rabbit muscles, which is compatible with Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (containing aluminum) (batch number: 202003002) had basically the same response; since the dose of aluminum hydroxide adjuvant in this trial was 1.0 mg/rabbit, the dose was twice the human dose, so after 14 days of the recovery period (another 1/2 rabbits continued to observe for 14 days after the necropsy was stopped 48 hours), the irritation lesions at the administration site were not significantly repaired.

4.3.2.3.2. Animal allergy testing

Forty Guinea pigs, male and female, sensitized by intramuscular injection, once every other day, 3 times in total; intravenous injection of 2 times the sensitizing dose to challenge; set up 5 groups: negative control group, positive control group, adjuvant control group (1 human dose), low-dose group (0.2 human dose), high-dose group (1 human dose), observe the systemic allergic reaction after challenge.

Under the dosage conditions of this test, the high-dose group of Recombinant Novel Coronavirus Vaccine (CHO cells) (batch number: 202004003) had a certain active systemic allergic reaction to guinea pigs, which basically disappeared within 7 minutes after injection, and there was no death of guinea pigs; There was no active allergic reaction in the dose group and the negative control group; the Recombinant Novel Coronavirus Vaccine (CHO cell) placebo (batch number: 202003002) had no active allergic reaction to guinea pigs.

For the high-dose group, the guinea pig's immunization dose was 1 human immunization dose, and the low-dose group was 0.2 human immunization dose. For this allergic reaction, the high-dose group had a certain active systemic allergic reaction, while for the low-dose group, no active anaphylaxis was seen, so it can be judged that the active anaphylaxis in the high-dose group is an allergic reaction caused by excessive immune adjuvant aluminum hydroxide.

4.4. Previous clinical studies results

4.4.1. Recombinant Novel Coronavirus Vaccine (CHO cell) Phase I clinical trial

The Phase I clinical trial of the Recombinant Novel Coronavirus Vaccine (CHO cell) in healthy people aged 18 to 59 years old is a multicenter, double-blind, randomized, placebo-control, dose escalation study. The investigational vaccine groups include a low dose group (25 μg/ 0.5ml/vial) and a high dose group (50μg/ 0.5 ml/vial).

A total number of 50 (20 in the low-dose vaccine group; 20 in the high-dose vaccine group; and 10 in the placebo group) subjects were enrolled. Subjects received one dose of the investigational vaccine or placebo at 0, 1, and 2 months respectively. At the first Phase of the study，25 subjects were randomly assigned to either the low-dose group (20 cases) or the placebo group (5 cases). 7 days after the subjects from the first Phase received the first dose, the DSMB conducted safety assessment, confirmed the safety and agreed to continue the trial and then the second Phase of the study was started and another 25 subjects were randomly assigned to either the high-dose group (20 cases) or the placebo group (5 cases). The safety of each subject is observed from the first dose of vaccination to 1 year after full vaccination so as to evaluate the safety and tolerability of the vaccine, during which blood samples are taken several times to preliminarily explore the immunogenicity of the vaccine.

The database of Phase I trial was locked and analyzed on October 22, 2020. The scope of database lock-in included the visit data and partial immunogenicity data collected by EDC before October 21, 2020.

The preliminary safety results of this study show that there is no significant difference between the high-dose group, low-dose group and placebo group in the overall incidence of adverse events and the incidence of adverse events related to the Investigational product among the groups. It can be seen that there is no significant difference in the incidence of adverse events between the investigational vaccine with different immunization dosages and the placebo. The incidence of adverse reactions at the site (local) of high and low dose vaccine is higher than that of placebo, but there is no statistical significance among the groups. After injection, the systemic and local reactions were mostly mild, transient, and the severity was mainly grade 1 and 2, and there was no serious adverse event related to the vaccine. At the same time, the

preliminary immunogenicity results of this study show that the positive conversion rate of live virus neutralizing antibody and the positive conversion rate of RBD binding antibody reached 100% in high and low dose groups after 7 days of injection.

The results of phase I clinical study showed that the safety and immunogenicity of the vaccine were good, and it could enter into the next large-scale clinical trial.

4.4.2. Recombinant Novel Coronavirus Vaccine (CHO cell) Phase II clinical trial

The Phase II clinical trial of the Recombinant Novel Coronavirus Vaccine (CHO cell) in healthy people aged 18 to 59 years old is a randomized, double-blind, placebo control study. The investigational vaccine groups include a low dose group (25 μg/ 0.5ml/vial) and a high dose group (50μg/ 0.5 ml/vial).

This trial includes healthy adults aged 18-59 with a total number of 900. There are 2-dose schedule (immunization at month 0, month 1) group (150 in the low-dose vaccine subgroup; 150 in the high-dose vaccine subgroup; 150 in the placebo subgroup) and 3-dose schedule (immunization at month 0, month 1 and month 2) group (150 in the low-dose vaccine subgroup; 150 in the high-dose vaccine subgroup; and 150 in the placebo subgroup). Blood samples of all subjects are collected before each dose, 14 days after each dose, 1 month, 6 months and 12 months after the full course of immunization for humoral immunogenicity analysis. Blood samples of the first 24 subjects in each group are collected before the first dose of inoculation, 4 days and 12 months after the full course of immunization for cellular immunogenicity analysis, and immunogenicity indexes of the two groups are compared. All subjects are also observed for safety from receiving the first dose of vaccine until 12 months after full course of immunization and are observed for immune persistence during 12 months after full immunization.

The database of Phase II trial was locked and analyzed on October 22, 2020. The scope of database lock-in included the visit data and partial immunogenicity data collected by EDC before October 21, 2020.

The preliminary immunogenicity results show that the positive conversion rate of live virus neutralizing antibody, GMT of live virus neutralizing antibody, positive conversion rate of RBD protein binding antibody and GMT of RBD protein binding antibody are all increased after injection, and the increasing trend became more

obvious with the increase of inoculation times.The levels of the above indicators on the 14th day after the third dose of investigational vaccine were significantly higher than those on the 14th day after the second dose of investigational vaccine, while the placebo group was consistent with that before immunization and maintained at a lower level, and the trend of the low and high dose group was roughly the same. On the 14th day after the third dose of vaccine, the above immunogenicity related indicators in the low-dose group were significantly higher than those in the high-dose group.

The preliminary safety results of this study show that: there were no deaths and serious adverse events related to the vaccine. The systemic adverse reactions were mainly fever and fatigue, and the local adverse reactions were mainly pruritus, erythema and swelling of the inoculation site. Most of them were mild and transient adverse reactions. No case of SUSAR occurred, which proved that the vaccine has good safety.

4.5. Rationale for the selection of vaccine dosage and immunization schedule

basis for selection of dose and immunization schedule

The analysis of immunogenicity in the currently completed phase II clinical trial for this vaccine showed that the positive conversion rate of neutralizing antibody of live virus and GMT of neutralizing antibody of live virus were higher 14 days after vaccination of three doses at low dose as compared with two-dose groups (low dose, high dose and placebo), three-dose groups of high dose and placebo. It was indicated that low dose (25 μg /0.5 mL/vial) should be selected for the phase III clinical study on this product, given for three doses according to 0, 1 and 2-month immunization schedule.

The interim analysis of safety data in the currently completed phase I and II clinical trials for this vaccine showed that local and systemic adverse reactions occurred in the subjects participating in the trials, the systemic adverse reactions were mainly pyrexia, headache and asthenia and so on, local adverse reactions were mainly pain, swelling, induration and flushing, which were all slight and transient, and grade 1 and 2 in severity, no death or vaccine related serious adverse events occurred in the trials, showing a good safety profile for each dose and immunization schedule of this vaccine.

In summary of the above immunogenicity and safety findings, low dose (25 μg /0.5

mL/vial) was selected and three-dose immunization schedule on Month 0, 1 and 2 was selected as the vaccination regimen for the phase III clinical trial for this vaccine.

4.6. Benefits/potential risks of subjects

4.6.1. Known potential risks

Participation in this study may prevent the respiratory disease induced by novel Coronavirus infection (COVID-19), and similar with any other vaccine, its immune effect needs to be evaluated in clinical trials; meanwhile, a part of subjects will receive placebo in this study, i.e., they will not have the protection from COVID-19, thus they may suffer from it for natural infection of novel Coronavirus during the observation.

At the same time, in some circumstances, the antibody may play a role in enhancing virus infection during viral infection, assist virus in entry into target cells and improve infection rate, this phenomenon is known as antibody dependence enhancement (ADE). In the last century, admission for vaccine enhanced disease (VED) was found in 80% subjects in the clinical trial on respiratory syncytial virus vaccine, and two subjects died finally. As a higher pathological injury score in lungs was observed after vaccination as compared with placebo group in the preclinical animal study (rhesus monkeys) for SARS vaccine, suspected or confirmed COVID-19 cases need to be closely noted in this clinical study. Subjects must be complaince with the local prevention and control requirements and go to hospital for diagnosis and treatment if they are suspected or confirmed to be infected with SARS-COV-2 during the study.

The potential risks for vaccination of Investigational product are only limited to the common adverse reactions of any vaccine for injection, for example, mild pain at the injection site and occasionally mild to moderate flushing, swelling and induration. Fever and anorexia may also occur, however, they are expected to be mild. Generally, they will relieve and disappear spontaneously, without treatment; the reactions may severe (e.g., high fever, allergic reaction, etc.) in individual subjects, which will be closely observed by investigators and undergo symptomatic treatment.

Blood collection is needed during the study, pain or ecchymosis may occur after blood collection.

4.6.2. Known potential risks

Subjects may get potential protection for vaccination of Investigational product, i.e., prevention from the respiratory disease induced by novel Coronavirus infection (COVID-19). Subjects will make contributions to the early approval of COVID-19

vaccine and benefit wider population through participation in this registration study.

4.7. Relevant definitions

(1) "Month" in visit: defined as "30 days".

(2) Woman at childbearing age: defined as the woman who is in a specific period from maturity of female reproductive organs (menarche) to ovarian failure (menopause).

(3) 18-59 years old: 18 years old (i.e., the date of 18 years old) and less than 60 years old (i.e., one day before 60 years old) on the day of enrollment.

(4) 60 years old and above: 60 years old (i.e., the date of 60 years old) and above on the day of enrollment.

5. Study Objectives and Endpoints

5.1. Study objectives

5.1.1. Primary objective:

To evaluate the protective potency and safety of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in the population aged 18 years and above.

5.1.2. Secondary objectives:

(1) To evaluate the protective potency of recombinant COVID-19 vaccine (CHO cell) in prevention of severe and critical COVID-19 in the population aged 18 years and above.

(2) To evaluate the immunogenicity and immune persistence of recombinant COVID-19 vaccine (CHO cell) in the population aged 18 years and above.

(3) To evaluate the protective potency of emergency vaccination of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in the population aged 18 years and above.

(4) To evaluate the protective potency of recombinant COVID-19 vaccine (CHO cell) in prevention of any grade of COVID-19 in different age groups (18-59 years old, 60 years old and above).

5.1.3. Exploratory objectives:

To explore the immunological surrogate variables of recombinant COVID-19 vaccine (CHO cell) in prevention of COVID-19 in the population aged 18 years and above.

5.2. Study endpoints

5.2.1. Primary study endpoints:

(1) Study endpoint of protective potency:

Number of patients with any grade of COVID-19 7 days after full course of vaccination.

(2) Safety endpoints:

1) Analysis of adverse events from the first dose of vaccination to 30 days after full course of vaccination: incidence of adverse reactions and adverse events; incidence of grade 3 and above adverse eventsand above adverse reactions; incidence of adverse events or adverse reactions leading to withdrawal.Analysis of serious adverse events from the first dose of vaccination to 12 months after full course of vaccination: incidence of serious adverse events; incidence of serious adverse events related with the Investigational product

5.2.2. Secondary study endpoints:

(1) Study endpoint of protective potency:

1) Number of patients with severe and severity above COVID-19 7 days after full course of vaccination;
2) Number of patients with any grade of COVID-19 7 days after the first dose of vaccination;
3) Number of patients with any grade of COVID-19 7 days after full course of vaccination in different age groups (18-59 years old, 60 years old and above).

(2) Study endpoints of immunogenicity and immune persistence:

SARS-COV-2 neutralizing antibody, RBD protein binding antibody IgG level on Day 14 , 6 months after full course of vaccination.

5.2.3. Exploratory endpoints:

SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG protective level against COVID-19 induced by novel Coronavirus infection.

6. Study Design

6.1. Overall design

A randomized, double-blind, placebo-controlled international multicenter clinical trial design will be adopted. A total of 29000 subjects aged 18 and above will be recruited, including 750 subjects aged 18-59 and 250 subjects aged 60 and above in China;

21000 subjects aged 18-59 and 7000 subjects aged 60 and above will be recruited outside China. Safety and immunogenicity will be evaluated in Chinese subjects, and protective efficacy, immunogenicity and safety will be evaluated in subjects outside China. Among them, 750 subjects aged 18-59, 250 subjects aged 60 and above and all subjects in China will be selected as immunogenicity subgroup. There will be 1000 cases in total, 500 cases in each group will be vaccinated with the investigational vaccine or placebo (the subjects in the immunogenicity subgroup participated in the evaluation of protection efficacy); meanwhile, an immunogenicity research cohort of 1000 cases will be set up in China, with 500 cases in each group being vaccinated with the investigational vaccine or placebo. The levels of neutralizing antibody of SARS-COV-2 and IgG of RBD protein binding antibody will be detected before immunization, 14 days after the whole immunization, and 6 months after the whole immunizationto evaluate the immunogenicity and immune persistence

The study will follow the requirements in ICH-GCP, subjects need to be informed of the background, content, potential risks and benefits of the study prior to the start of the study. Subjects need to provide written informed consent form, investigators should use local language to explain the risks and benefits of the study to subjects, including potential side effects of the vaccine. Subjects will also be told to provide corresponding biological samples for testing. Subjects also need to provide their own clinical data to investigators, however, these data will be confidentially saved and processed in accordance with local national and international criteria and regulatory requirements.

Screening eligible subjects will obtain one study number and be randomized to placebo control group or Investigational product group. The vaccination will be performed in accordance with Month 0, 1,2 vaccination procedure at the dose of 25μg/0.5mL/vial , and the follow-up visit will continue for 14 months.

6.2. Criteria on study suspension or termination

Acquisition of new data on the Investigational product from this study or any other study that is considered by regulatory authorities, the sponsor, investigators and/or institutional review board /Independent Ethics Committee as requiring suspension/termination of the study;

Criteria on study suspension:

In case of the following conditions, the study needs to be suspended, and the institutional review board/independent ethics committee, relevant regulatory

authorities will be reported immediately, data safety monitoring board (DSMB) expert meeting will be held urgently for safety demonstration, analysis and determination on whether to continue this study.

Adverse event leading to study suspension	**Number/%**
Vaccine-related deaths or serious life-threatening adverse reactions occurred during the study period	⩾1 case
≥grade 3 adverse reaction for 48 hours, following any dose-time of vaccination	>15% vaccinated persons

Criteria on study termination:

In case of the following conditions, the study needs to be terminated, and the institutional review board/independent ethics committee, relevant regulatory authorities will be reported immediately.

Adverse event leading to study termination	**Number/%**
≥grade 3 adverse reaction for 48 hours, following any dose-time of vaccination	>30% vaccinated persons

If the study is suspended or terminated prematurely, the sponsor will inform investigators, the ethics committee and drug regulatory authorities of the reason for the suspension or termination immediately in accordance with the requirements in corresponding registration regulations.

Regardless of the reason for early termination of the study, investigators should inform subjects immediately, and ensure appropriate follow-up of subjects.

6.3. ADE/VED (antibody dependence enhancement/ vaccine enhanced disease) risk monitoring

Following vaccination (at least one dose of Investigational product). If the subject is diagnosed as COVID-19, he/she should be hospitalized or isolated in accordance with requirements for epidemic prevention and control in that region. Special investigation is needed for severe or critical dead cases, and DSMB will analyze the presence of ADE/VED according to the results of investigation.

7. Monitoring of COVID-19 case

7.1. Diagnostic criteria and definition

(1) Suspected COVID-19: Those who meet any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat,

stuffiness, headache, diarrhea, anorexia/ nausea/emesis, loss of smell/taste .

(2) COVID-19: Suspected cases were sampled twice (two samples were collected for each time, one for testing and one for backup storage) , at a sampling interval of 24-48h. The result of Real-time fluorescence quantitative RT-PCR test for SARS-COV-2 nucleic acids was positive at least once and having any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia/nausea/vomiting, loss of smell/taste.

(3) Mild COVID-19: Confirmed COVID-19 without evidence on viral pneumonia or hypoxia, Having symptoms include fever, cough, fatigue, anorexia, shortness of breath, myalgia, sore throat, stuffiness, headache, diarrhea, nausea, vomiting, loss of smell (anosmia), loss of taste (ageusia).

(4) Moderate COVID-19: Confirmed COVID-19, having clinical signs of pneumonia (fever, cough, dyspnea, shortness of breath) but no signs of serious pneumonia, including SpO2≥ 90% under indoor air conditions.

(5) Severe or critical COVID-19: including a) and b)

a) Severe COVID-19: Confirmed COVID-19 and meet any of the following conditions:1) shortness of breath, RR ≥ 30 times / min; 2) under resting state and indoor air condition, SpO2＜90%; 3) arterial partial pressure of oxygen (PaO2) / oxygen inhalation concentration (FiO2) ≤ 300 mmHg (1mmhg = 0.133kpa); High altitude (above 1000 m) should be corrected for PaO_2 / FiO2 according to the following formula: PaO2/FiO2 x [760/atmospheric pressure (mmHg)]; 4) the clinical symptoms worsened progressively, and the lung imaging showed that the lesions progressed more than 50% within 24 to 48 hours.

b) Critical COVID-19: Confirmed COVID-19, with symptoms include respiratory failure (high-throughput oxygen therapy, non-invasive ventilation, mechanical ventilation, ECMO), shock, admission to ICU, sepsis, complicated with acute pulmonary embolism, complicated with acute coronary syndrome, complicated with acute stroke, complicated with delirium, complicated with other organ failure, death, etc.

7.2. Case monitoring, diagnosis and treatment

7.2.1. Diagnostic procedure for suspected cases

Symptom-driven passive monitoring is used for monitoring of COVID-19 cases. Prior

to the start of the study, investigators should establish all kinds of feasible communication channels, including network, telephone, text message, face-to-face interview, make sure the subjects can communicate with investigators at any time, be familiar with the monitoring and diagnostic procedure of COVID-19 cases, and implement personal protection strictly. Develop a unified standard operating procedure (SOP) for all novel Coronavirus nucleic acid testing methods and train investigators on all methods of detection of Novel Coronavirus nucleic acid to ensure the authenticity and reliability of results and reduce differences.

After first dose of vaccination, the following procedure will be initiated if the subject has the relevant symptoms, which are included in the definitions of suspected COVID-19 cases.

1. Subjects will contact investigators immediately (face-to-face interview or telephone, text message, network, etc.).
2. Investigators will confirm if the definition of suspected case is met based on the symptoms provided by subjects and fill in case surveillance form.

   (1) If yes, subjects will arrive at the study institutions or designated medical institutions as early as possible for the following examinations, as instructed by investigators. Two samples of nasopharynx swabs will be collected by on-site investigators(two samples will be collected for each sampling). One of the samples should be detected by real-time fluorescent quantitative RT-PCR, and the other should be kept as a backup. Respiratory rate (RR), oxygen saturation (SpO2), oxygenation index (PaO2 / FiO2) will be performed. The investigators will collect the RT-PCR test reports, diagnosis source file, mutations of S gene test report and related inspection report sheet of all subjects with positive results in PCR tests:

   a. If the result of RT-PCR test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be managed according to the local epidemic diagnosis and treatment measures, and samples were taken again for testing within 24-48 hours. Meanwhile, ARMS-PCR should be employed to detect the mutations of S gene of novel Coronavirus, mainly including N501Y, A570D, HV69-70del, K417N, K417T and E484K..

   b. .If the result of RT-PCR test is negative, samples were taken again for

testing within 24-48 hours, and the testing of respiratory rate (RR), oxygen saturation (SpO2) and oxygenation index (PaO2/FiO2) was performed.

a) If the result of re-sampled RT-PCR test is positive, the investigators shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. Meanwhile, the mutations of S gene of novel Coronavirus should be detected..

b) If the result of re-sampled RT-PCR test is negative, the investigators will follow up the subjects. If the original symptoms continue or worsen or new related symptoms of suspected cases appear within 2 days, the subjects will be monitored according to the content specified in "(1)" and sampled again for testing. The follow-up can be discontinued if the original symptoms of the subjects disappear and no new related symptoms of suspected cases occur during the follow-up.

In a patient with suspected COVID-19, especially with severe illness, a single negative upper respiratory tract (URT) sample does not exclude the diagnosis, and additional URT and lower respiratory tract (LRT) samples (sputum, endotracheal aspirate, or alveolar lavage) are recommended. LRT (vs URT) samples are more likely to be positive and last longger. The investigators could elect to collect only LRT samples when these are readily available (for example, in mechanically ventilated patients).

(2) Otherwise investigators will provide advice, for example, routine medical treatment.

7.2.2. Judgment for severity of COVID-19

For the subjects who are diagnosed as COVID-19, they should be treated and cured at the study institutions or designated medical institutions as far as possible according to local therapeutic regimen if hospitalized, investigators should collect the clinical data of relevant examinations and diagnoses during hospitalization (see Appendix C)and record in the case surveillance form; if not hospitalized, subjects shall be treated according to the local epidemic diagnosis and treatment measures, and home care should be taken to take personal and family epidemic prevention measures, followed up by investigators through face-to-face interview, telephone, text message or

network everyday, as to collect the data on the course of disease (including start time and end time of each symptom, whether new symptoms, etc.) , until its outcome and record in the case surveillance form. If the subject has difficulty breathing during the follow-up, the investigator should remind the subject to go to the hospital immediately for treatment

The severity will be judged based on the data on the course of disease or the data consistent with the criteria on critical illness by the investigators (note: subjects without critical illness need to be followed up until its outcome, the severity will be judged in accordance with the most serious point in the course of disease in combination with the definition of severity of COVID-19, if the subject has critical illness, i.e., reaching the judgment point, the severity can be judged with no need to wait for its outcome).

Start time of disease: the occurrence time of the first symptom or sign at the first diagnosis of COVID-19 in laboratory. For example, one subject reports cough and fever to investigators on August 1, COVID-19 is diagnosed in the laboratory on August 3, the start time of disease should be recorded as August 1 for this subject.

End time of disease: the time when the disease is cured (based on the criteria on cure in each country); in case of death, the time of death will be used as the end time; if the subject still can not be cured at the end of the study, the end time will be left blank and the outcome will be recorded as “still ongoing”.

7.2.3. Review of COVID-19 case

All the clinical data and case report form from the cases diagnosed as COVID-19 will be submitted to EAC in accordance with the time points and procedure specified in Endpoint Adjudication Committee (EAC) constitution, EAC will check COVID-19 cases and the severity of COVID-19, the final results will be based on the evaluation by EAC.

7.2.3.1 Confirmation review of COVID-19 cases

The investigators shall collect clinical data from all subjects who are positive for PCR tests and submit the data to the Endpoint Determination Committee (EAC), which will review the COVID-19 case in accordance with the procedure set forth in the EAC's statutes. At the same time, under the premise of conforming to the policies of target countries, an independent third party can be invited to review the positive samples in response to review requirements. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.

7.2.3.2 Confirmation review of the severity of COVID-19 cases

The investigators shall collect all clinical data from the follow-up visits of confirmed COVID-19 cases and submit the data to the EAC, which will review the severity of the COVID-19 case in accordance with the procedures set forth in the EAC's protocols. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.

7.3. Time of case monitoring

All the subjects receiving the vaccination will be monitored for COVID-19 during the study. The data on COVID-19 monitored from the 1st dose of vaccination to one year after the full course of vaccination will be statistically analyzed, and used for the registration application of the vaccine.

7.4. Collection of biological samples

1) Nasopharyngeal swab biological sample will be collected from the subjects with suspected COVID-19 reported at each study site (based on the SOP for collection of biological samples).
2) The biological sample collected at each site will be divided in duplicate, one for real-time fluorescence quantitative RT-PCR and/or detection of mutations of S gene of novel Coronavirus at the laboratory for detection of novel Coronavirus nucleic acid (based on the SOP for detection of novel Coronavirus nucleic acid and the SOP for detection of mutations of S gene of novel Coronavirus); the other one archived and stored in qualified laboratory for back-up.

8. Study Population

8.1. Study subjects

Study subjects are ≥18 years old.

8.2. Inclusion criteria

(1) Subjects ≥18 years old;
(2) Subjects who voluntarily participate in the study and sign the informed consent form, and can provide the effective identity certificate, understand and follow the requirements of the study protocol;
(3) Female subjects of childbearing age who agree to use the effective contraceptive measures from the starting of the study to 6 months after the completed inoculation.

8.3. Exclusion criteria

(1) Suspected or confirmed as fever (axillary temperature ≥37.3°C / oral temperature ≥37.5°C) within 72 hours before the enrollment, or axillary temperature ≥37.3°C / oral temperature ≥37.5°C at the day of screening;

(2) Diastolic blood pressure ⩾ 100mmhg and / or systolic blood pressure ⩾ 150mmhg;

(3) Patients with previous history of a COVID-19;

(4) Detection of SARS-COV-2 nucleic acid or antibody is positive;

(5) Those who are suffering from the following diseases:

① With thrombocytopenia, any coagulation dysfunction or receiving anti-coagulatory treatment

② Congenital or acquired immune deficiency or autoimmune disease history; no spleen, or history of splenic surgery and trauma, or receiving immunomodulator treatment within 6 months, e.g., immunosuppressive dose of glucocorticoids (reference dose: equivalent to 20mg/ day of prednisone, over 1 week); Or monoclonal antibodies; Or thymosin; Or interferon etc.; However, topical application (such as ointment, eye drops, inhalers or nasal sprays) is permitted;

③ Symptoms related to acute respiratory tract infection (such as sneezing, nasal congestion, runny nose, cough, sore throat, loss of taste, chills, shortness of breath, etc.);

④ Cancer patients (except basal cell carcinoma)

(6) With a history of serious allergy to any vaccine or any composition of Investigational product (including: aluminum preparations), such as allergic shock, allergic throat edema, allergic purpura, thrombocytopenic purpura, localized allergic necrosis reaction (Arthus reaction), dyspnea and angioneuroedema;

(7) Inoculated with subunit vaccine and inactivated vaccine within 14 days before the first dosing of investigational? vaccine, or inoculated with attenuated live vaccine within 30 days;

(8) Previous receiving blood transfusion or blood relevant products (including immunoglobulin) within 3 months, or planning to receive such products from the starting of study to <6 months after the whole-course inoculation;

(9) Have participated in or are participating in other covid-19 related clinical trials;

(10)Women in breastfeeding period or in pregnant period (including women at childbearing age with positive result of urine pregnancy test);

(11) Considered by investigators as any disease or state possibly making the subject at unacceptable risk; not conforming to the requirements of study protocol; interference of assessment of reactions of vaccine.

9. Study Procedures

Subjects must read and sign an informed consent form approved by the Ethics Committee prior to the study. Each examination and study procedure are performed in accordance with the study flowchart.

9.1. Informed consent

The subjects must sign the latest approved version of informed consent from with noting a date before any study procedure is performed.

The contents of ICF include at least: overview of clinical trial; objective of trial; tentative contents involved in clinical trial; investigational treatment and possibility for random allocation into each group; procedures of trial to be observed by subjects; predicted benefit of trial; and possibility for unable benefit., risks of subjects; expenses to be paid; compensation; scope of use and confidentiality of samples

The subject can withdraw from study for any reason and at the same time, their legitimate rights and interests shall not be affected in any way.

The subject will possess sufficient time to consider such information and have an opportunity to inquire investigators or other independent party for deciding on whether to participate in this study. The investigators must possess the relevant qualification and experience, and obtain the authorization of PI. Then, ICF is jointly signed by investigators and subjects, which is in duplicate. A copy of the signed ICF will be given to the subject. The signed original is kept in study site.

9.2. Evaluation of inclusion and exclusion criteria

Visit 1 (D-7~0): After ICF of latest approved version is signed by subjects, screening number is assigned by investigators; demographic data are collected (sex, birth date, race,height and weight); medical history (past illness and present illness), allergic history, recent medication (vaccine) history and concomitant medication medication are inquired, physical examination (skin and mucous membranes, lymph nodes, head, neck, chest, abdomen, spine / limbs)axillary temperature is measured; nasopharyngeal swab is collected for nucleic acid test on SARS-COV-2 (real-time fluorescence

quantitative PCR, RT-PCR),;blood sample is collected for antibody test on SARS-COV-2 (colloidal gold technique); inclusion/exclusion criteria are verified.

Visit 2 (D0): concomitant medication(If visit 1 and visit 2 are not on the same day), and perform vital signs assessment (blood pressure, axillary / oral temperature, pulse) are inquired by investigators again; axillary temperature is measured; in female subjects at childbearing age, urine pregnancy test is made. According to the inclusion/exclusion criteria, the subjects are assessed by investigators; Determine the subject who meets the criteria

9.3. Randomization and blinding

9.3.1. Randomization

Stratified block randomization is used in this trial, subjects are stratified by center (participating country) and age (18~59 years, 60 years and above), randomized allocation of subjects and Investigational product are completed using Interactive Web Response System (IWRS).

SAS 9.4 or above version software is used by randomization statisticians to generate subject randomization table and vaccine randomization table, which are imported into IWRS system by system engineer. Upon successful screening of subjects, the study personnel participating in this trial at each site will login IWRS system to acquire subject's random number; investigators will login IWRS system to acquire vaccine number prior to vaccination, and immunize according to vaccine number; in case of vaccine damage, investigators can acquire a new vaccine number from IWRS system and perform vaccination with the new vaccine number.

9.3.2. Blinding

Prior to the start of the study, the staff from the sponsor who are not involved in this clinical study will blind the Investigational product uniformly together with non-blind randomization statisticians, i.e., paste the printed label at the designated position of each vaccine according to the content of vaccine blindness. The blinding of vaccine will be supervised by randomization statisticians, who will guide blinding operators to label according to the content of blindness. Upon completion of blinding, the content of blindness should be sealed by non-blind randomization statisticians. The whole blinding process will be recorded and written in document form, i.e., blinding record, and kept as one of the important documents for this clinical study. The blinding personnel must not be involved in other relevant work in this clinical study or reveal the content of blindness to anyone who is involved in this clinical study.

This study includes one subject randomization table and one vaccine randomization table, the content of blindness including the parameter encoding corresponding group and the number of seed generating random number is sealed in duplicate and handed over to the sponsor and the unit in charge of the clinical study for preservation

respectively, and should be kept properly until lock of database.

9.3.3. Emergency unblinding

In case of emergency, when the investigator considers knowing the vaccine used by the subject is conducive to the management of adverse events, the detailed group of the subject can be obtained through the emergency unblinding module in IWRS system. In case emergency unblinding is needed, relevant personnel from the sponsor must be notified in advance as far as possible prior to the unblinding of the investigational vaccine, if possible. In case it fails to contact the sponsor prior to emergency unblinding, the sponsor must be contacted within 24 hours after emergency unblinding. Corresponding cases will be processed as drop-out once emergency unblinding is performed. Investigators need to record the date and reason for unblinding, and the unblinding process in the source document.

9.3.4. Unblinding requirements

When all the data are entered and signed, and the analysis population is determined in the blind data review meeting, the database will be locked. Unblinding can be carried out only upon lock of database, the vaccine corresponding to the study number will be unblinded. The unblinding document will be signed jointly by the principal investigator, sponsor and statisticians.

9.4. Vaccination

Visit 2 (D0): After the subjects passing the screening are grouped randomly, the vaccine or placebo is injected intramuscularly into deltoid muscle at upper arm. In order to ensure the safety of subjects, the subjects are retained for observation of 30 minutes after the inoculation; and all adverse events occurring during this period are recorded by investigators.

Before the subjects leave study field, the subjects are trained by investigators on the completion of diary card; straight ruler and thermometer is granted; the subjects are guided to measure axillary temperature and oral temperature through the straight ruler and thermometer; and the reactions at the site of injection are recorded. After the subjects leave study field, the axillary temperature, concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 0~7 days after the inoculation are recorded according to relevant requirements; At the same time, notified cards will be issued to the subjects, including the symptoms related to the suspected cases and the contact information of the investigators. The investigators will explain to the subjects. If the symptoms in the reminder card appear after the subjects leave the site, they need to contact the investigators in time, and the

investigators will give advice on whether they need to go to the institute or the designated institution for COVID-19 nucleic acid detection, if the subject left the test site, otherabnormal conditions need be contacted in time after the subjects leave study field, the adverse events occurring after this inoculation and the suspected cases of COVID-19 are treated by investigators in time; original medical record is completed.

At Day 0 of study, in the immunogenicity subgroup of subjects passing the screening, 5 mL of blood sample is additionally collected before the inoculation for immunogenicity test.

9.5. Follow-up

Concrete information on follow-up is shown in Attachment A: Study procedures for subjects.

Visit 3 (V2+8): The diary card is collected by investigators; the contents of diary card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Contact card is granted by investigators to subjects; the subjects are trained on the completion of contact card; the contact card is used to record the concomitant medications and adverse events (unsolicited AEs) occurring at 8~30 days after the inoculation.

Visit 4 (V2+30): The contact card is collected by investigators; the contents of contact card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Axillary temperature/oral temperature of subjects is measured. After the assessment and confirmation of investigators, the vaccine or placebo is inoculated for the second dosing into subjects. In order to ensure the safety of subjects, the subjects are retained for observation of 30 minutes after the inoculation; and all adverse events occurring during this period are recorded by investigators. Before the subjects leave study field, the diary card is granted; the axillary temperature and /oral temperature, concomitant medications and adverse events (including solicited and unsolicited AEs) occurring at 0~7 days after the inoculation are recorded.

Visit 5 (V4+8): The diary card is collected by investigators; the contents of diary card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed. Contact card is granted by investigators to subjects; the contact card is used to record the concomitant medications and adverse events (unsolicited AEs) occurring at 8~30 days after the inoculation.

Visit 6 (V4+30): The investigator collected the contact cards, reviewed the contents of the contact cards and signed for confirmation, evaluated the adverse events and filled in the original medical records. The axillary temperature / oral temperature of the subjects will be detected, and the subjects will be given the third dose of vaccine or placebo after the evaluation and confirmation of the investigators. To ensure the safety of the subjects, the subjects will be observed for 30 minutes after injection, during which all adverse events occurred within 30 minutes after vaccination were recorded by the investigators. A diary card will be issued before the subjects left the site to record the axillary / oral temperature, concomitant medication and adverse events (both the solicited and the unsolicited adverse events) at 0-7 days after injection.

Visit 7 (V6 + 8) - the investigator collected the diary card, reviewed the contents of the diary card and signed for confirmation, evaluated the adverse events and filled in the original medical record. A contact card was issued to the subjects to record the drug combination and adverse events (non solicitation adverse events) 8-30 days after vaccination.

Visit 8 (V6+14): In the immunogenicity subgroup of subjects, 5 mL of blood sample is collected for immunogenicity test.

Visit 9 (V6+30): The contact card is collected by investigators; the contents of contact card is verified and confirmed through signature; the adverse events are assessed; and original medical record is completed.

Visit 10 (V6+180): In the immunogenicity subgroup of subjects, 5 mL of blood sample is collected for immunogenicity test.

Visit 11 (V6+360): Telephone visit, serious adverse events occurring from the inoculation for first dosing to <12 months after the whole-course inoculation are collected by investigators.

9.6. Concomitant medications

At each visit/contact, the subjects are inquired by investigators on whether any drug has been given and any vaccine has been inoculated. All concomitant medications/vaccines are recorded (except vitamin and/or food additives). The concomitant medications are transcribed by investigators into eCRF.

Combined drugs mean all drugs (except Investigational product) given into subjects from the signing of ICF to <30 days after the inoculation for last dosing and all drugs (except Investigational product) given due to the SAE and pregnancy from 30 days to

1 year after the inoculation for last dosing, including: antibiotics, antiviral drugs, antipyretic analgesics, anti-allergic drugs, biological products (vaccine), and Chinese (patent) medicines (except vitamin and/or food additives).

The concomitant medications are classified by data administrator into the following 10 categories:

(1) Hormones/steroids and other immunosuppressants;
(2) Antiallergic drugs;
(3) Antipyretics/analgesics/non-steroidal anti-inflammatory drugs;
(4) Vaccines and biological products;
(5) Immune globulins and other blood products;
(6) Antibiotics;
(7) Antivirals;
(8) Chinese patent medicine;
(9) Recipe of Chinese medicine
(10) Others.

Allowable vaccine: The vaccine is used by observing the inclusion/exclusion criteria; for the emergency inoculation of vaccine (such as rabies or tetanus), such limitation should not be required, but the use conditions of vaccine should be recorded strictly by the facts according to the relevant requirements. Inoculation of other vaccines before the inoculation of Investigational product: The subunit vaccine and inactivated vaccine is inoculated by interval of at least 14 days from the inoculation of Investigational product; the attenuated live vaccine is inoculated by interval of at least 30 days from the inoculation of Investigational product.

Allowable medications: During the trial, necessary medications shall be allowed for treatment if the subject develops any adverse events, and the medication information shall be recorded according to the requirements. If contraception requirements are raised for subjects during study period, contraceptives can also be allowed; but the information on any used drug should be recorded strictly by the facts according to the relevant requirements.

Preventive drugs: mean the drug given when there are no symptoms or anticipated occurrence of vaccination reactions. If Aspirin is used by subjects for treating heart disease, the used Aspirin is a type of concomitant medications to be reported, but is not a type of preventive drugs. If antipyretics is given for fever prevention in the subjects without fever during the recruitment period, the antipyretics is considered as

a type of preventive drugs. At enrollment, the subjects are inquired on the ongoing drugs to confirm that antipyretics, analgesics or anti-allergic drugs are not given.

9.7. Immunogenic blood samples processing

For immunogenicity test, about 5 mL of blood sample is collected by avoiding a hemolysis. The centrifuged serum is subpacked into 4 freezing tubes (about 250 μL each tube), including: 3 tubes sent for examination and 1 tube for reservation; after the marking, these tubes are kept at -20°C (treatment procedures for biological sample are shown in the treatment SOP for biological samples).

9.8. Detection of immunogenic blood samples

The inspection quality control standard is provided by the National Institutes for Food and Drug Control. The test on SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG is completed by National Institutes for Food and Drug Control of China.

9.9. Withdrawal criteria

Subjects may withdraw from the study at any time. Besides, the investigator can require the subject to withdraw from the study at any time for following reasons:

- Occurrence of AEs or complicated conditions unable to continue the study;
- Participating in other clinical trials before the termination of this clinical trial;
- Subject s with other conditions that are not suitable to participate in the clinical trial, as considered by the investigator.

The reasons for study withdrawal are recorded into case report form (CRF); the withdrawn or rejected subjects will not be replaced.

9.10. End of study

The ending date of study in 1 year after the whole-course inoculation of vaccine in last subject.

10. Investigational product

10.1. Description of Investigational product

10.1.1. Recombinant Novel Coronavirus Vaccine (CHO Cell)

The Recombinant Novel Coronavirus Vaccine (CHO cell) prepared by Anhui Zhifei Longcom Biopharmaceutical Co., Ltd (“Zhifei Longcom”) is produced by recombinant CHO cell expressing the receptor binding region of the CoronavirusNovel Coronavirus spike glycoprotein (recombinant protein NCP-RBD) after purification, with aluminum hydroxide adjuvant added. The product can be

stratified by precipitation and after shaking, it is easy to disperse. “Novel Coronavirus Vaccine (CHO Cell) Manufacturing and Verification Regulations (Draft)” has passed the verification of both the company and China food and Drug Control Institute. The relevant information and content are as follows:

Name:	Recombinant Novel Coronavirus Vaccine (CHO cell)
Manufacturer:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd
Batch number:	See Quality Inspection Report
Specifications:	0.5ml/vial. It contains 25 μg NCP-RBD protein
Ingredients:	NCP-RBD Aluminum Hydroxide Adjuvant
Other:	See Quality Inspection Report
Inspection Institution:	National Institutes for Food and Drug Control
Inspection Report No.:	See quality inspection report
Shelf Life:	2 years(provisional)

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible institution shall explain and report to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.1.2. Novel Coronavirus Vaccine Placebo

The placebo, produced by Anhui Zhifei Longcom Biological Pharmaceutical Co., Ltd., does not contain any effective ingredients of the COVID-19 vaccine. The ingredients and contents are as follows:

Name:	Novel Coronavirus Vaccine (CHO cell) placebo (with aluminum)
Manufacturer:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd

Batch number:	See Quality Inspection Report
Specifications:	0.5 ml/vial. It contains 0.25mg aluminum hydroxide adjuvant
Ingredients:	Aluminum hydroxide adjuvant
Other:	See Quality Inspection Report
Inspection Institution:	National Institutes for Food and Drug Control
Inspection Report No.:	See Quality Inspection Report
Shelf Life:	2 years(provisional)

If the vaccine batches used in the trial are inconsistent with those recorded in the protocol, the responsible institution shall explain and report to the Ethics Committee (or according to the requirements of IRB) before the start of clinical trial.

10.2. Management of Investigational product

The institution in charge of vaccine clinical trial needs to guide the study site to formulate the management system of Investigational product, the management of receipt, safekeeping, recovery, return/destruction of Investigational product needs to meet the requirements in relevant SOP. The institution in charge of vaccine clinical trial and study site needs to designate the personnel trained on GCP and relevant knowledge to take charge of management of Investigational product.

Vaccine transportation: the full course of vaccine management needs to meet cold-chain requirements, vaccine transportation and storage conditions as required in the protocol must be available. The vaccine will be stored and transported at 2-8℃, protected from light, a delivery note and temperature monitoring must be available during vaccine transportation, the packaging and unpacking temperature will be recorded on arrival, the receiver will sign on the delivery note and fax or copy to the shipper upon verification of the vaccine, the delivery note will be kept properly by both parties.

Vaccine storage and distribution: the Investigational product will be stored in separate partition at 2-8℃, protected from light, managed according to the locking requirement by specially-assigned person in special counter, blind management needs to be maintained for blind study. The vaccine receiver must check the vaccine delivery status, establish worksheets of vaccine handover, registration, use and recovery, fill in it as required and keep in the work record.

Record of vaccine handover: the Investigational product will be provided by the sponsor, investigators will check the name, dosage and package of the vaccine whilst receiving them, and make a record of handover.

Record of vaccine registration and use: the record of vaccine registration and use will be formulated by investigators. The vaccines distributed to each subject should be well recorded, including the study number, subject initials and signature of vaccinator.

Record of vaccine retrieving: the vaccine administrator needs to recover the residual vaccines in time, place them separately, count and complete the inventory record on a regular basis, and the inconsistencies between the used vaccines and residual amount and the total number should be explained. The discarded, expired and residual vaccines in this study will be returned to the sponsor, the external packing of all the used vaccines needs to be retained for inspection during the study. The sponsor will verify the vaccine amount whilst receiving them and make relevant records well, which will be signed by vaccine administrator and the sponsor's representative. The sponsor must keep the vaccine at least until completion of NMPA verification according to cold-chain requirements.

Cold-chain failure: once the temperature for vaccine storage is <2℃ or >8℃, it will be regarded as cold chain failure. The sponsor needs to elucidate the information on vaccine stability in the investigator's brochure, once cold chain failure occurs, investigators should transport the vaccine to a dark environment at 2~8℃ for storage, use of the vaccines suffered from cold-chain failure should be suspended, and the sponsor should be reported as early as possible, discontinuation or continuation of the use of vaccine will be determined based on the written opinion from the sponsor. The vaccines suffered from cold-chain failure can not be used for subjects prior to acquisition of the sponsor's opinion on their disposal.

The investigational vaccine and control vaccine can not be used for non-clinical trial population.

11. Safety Report

11.1. Definitions

Adverse Events (AE)	Referring to any accidental medical accident occurred in the subject after he/she receiving the Investigational product treatment, including those accidents not necessarily caused by or related to the product.
Solicited Adverse Events	Adverse events collected as safety endpoints in the study, referring to the adverse events information collected by investigators or subjects during a specific follow-up period after injection.

Unsolicited Adverse Events	Other adverse events reported in the study other than solicited adverse events, also include those reported not during the designated solicited adverse reaction time window.
Adverse Reactions (AR)	Referring to any harmful or unexpected reaction that may be related to the Investigational product in clinical trial. There is at least one reasonable possibility, which can not be excluded, of the causal relationship between the Investigational product and the adverse event.
Serious Adverse Events (SAE)	Referring to the adverse medical events such as death or life-threatening conditions, permanent or serious disability or function loss, the need for hospitalization or extended hospital stay, and congenital abnormalities or birth defects after receiving the Investigational product.
Serious Adverse Reactions (SAR)	Referring to an adverse event that is both serious and considered to be an vaccine adverse reaction.
Suspected Unexpected Serious Adverse Reactions (SUSAR)	Referring to the suspicious and unexpected serious adverse reactions with the nature and severity that is exceeding the existing available information such as the investigator's brochure of the investigational vaccine, instruction of marketed drug or the synopsis of the product characteristics.
Anticipation	The investigator and the sponsor should determine whether the serious adverse events associated with the trial vaccine are expected or unexpected. If the nature, severity or frequency of the adverse event is inconsistent with the risk information of the previously described study intervention, it should be considered unexpected.

11.2. Correlation with Investigational product

For the expected or unexpected AE (solicited or unsolicited AE), the investigator should take measures to judge the correlation with vaccination in time, timely discover SAE and mass and tendentious adverse events related to vaccination in the

clinical trial, and timely suspend or terminate the clinical trial in order to minimize the harm to the subjects.

Definitely Relevant	There is clear evidence of causality, and other possible contributing factors can be excluded.
Highly Likely Relevant	There is evidence of a causality, and other factors are unlikely to be involved.
Possibly Relevant	There is some evidence of causality (e.g., the event occurs within a reasonable time after administration of the Investigational product). However, the influence of other factors may lead to the occurrence of the event (such as the clinical conditions of the patients, other concomitant treatment).
Possibly Not Relevant	There is little evidence of a causality relationship (e.g., the event did not occur within a reasonable time after administration of the Investigational product), or there is another reasonable explanation for the event (such as the clinical conditions of the subjects, other concomitant treatment).
Definitely Irrelevant	There is no evidence of any causality

11.3. Solicited adverse events

Solicited AE: The following events occurred within 7 days after injection:

Injection site (local) adverse events	Pain, swelling, induration, redness, rash, pruritus
Vital signs	fever
Non injection site (systemic) adverse events	Headache, fatigue / fatigue, nausea, vomiting, diarrhea, muscle pain, cough, acute allergic reaction, mental disorder (specific symptoms)

11.4. Unsolicited adverse events

Unsolicited adverse events are all adverse events, other than solicited adverse events, which reported during the period from the first dose of vaccination to 30 days

after the whole vaccination, and also include the adverse events reported outside the designated recruitment time window (for example, if the above-mentioned solicited adverse events occur on or after the 8th day of vaccination, it will be recorded as an unsolicited adverse event)

11.5. Recording procedure for adverse events

The AE grading standard of this study will be recorded and evaluated on the basis of the Guideline on the Classification of Adverse events in Clinical Trials of Preventive Vaccine issued by the National Medical Products Administration (NMPA) and in combination with the requirements of regions outside China .

During the trial, adverse events observed by investigators or reported by subjects will be recorded on the case report form (CRF), whether or not related to the investigational vaccine.

The following information will be recorded: the name of the adverse event, the date of onset and end, the severity, the evaluation of correlation between the AE and the vaccine, the concomitant medication and non-medication treatment, and the measures taken. Follow up information should be provided if necessary.

The adverse events assessed by qualified medical investigators as relevant to the investigational vaccine should be followed up until the events end or stabilize.

It is the investigator's clinical assessment to determine whether the severity of AE requires the subject to withdraw from trial. Subjects may also voluntarily withdraw from treatment due to adverse events that they consider intolerable. In case of any of the above conditions, the subjects must be assessed at the end of the trial and given appropriate care under medical supervision until the symptoms stop or the conditions stabilize.

11.6. Reporting of serious adverse events

11.6.1 Time of reporting

All the procedures for SAE reporting will be carried out by on-site PI in accordance with local/national ethics committee and regulatory requirements, however, the SAE should be reported to the sponsor within 24 hours. Upon receipt of safety related information, the sponsor should analyze and evaluate it immediately, including the seriousness, correlation with the investigational product and whether it is one expected event.

11.6.2 Content of report

1) Type and time of report (initial report, follow-up report, summary report and

corresponding time of report);

2) Subject's information (name initials, study number, date of birth, gender);

3) Reporter's information (name of medical institution and specialty, telephone, position / title);

4) Information on the suspected drug (Chinese and English names, registration classification and dosage form);

5) Study related information (clinical study approval letter number, clinical study classification, clinical trial indication);

6) Concurrent disease and therapy information (name of diagnosis, name and administration and dosage of therapeutic agent);

7) Detailed information on SAE (name of diagnosis, whether it belongs to ADE/VED, seriousness criteria, time of onset, end time, laboratory examination findings, course of therapy, prognosis, measures taken for the investigational vaccine and correlation with the investigational vaccine);

8) Unblinding;

9) Time of awareness by the investigator;

10) Signature of the investigator.

11.7. Suspected unexpected serious adverse reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) needs to meet the following three criteria at the same time:

1) Suspected adverse reaction: defined as the harmful reaction unrelated with the administration at any dose, its correlation with the drug is considered to be at least possibly related through analysis.

2) Unexpected adverse reaction: defined as an adverse reaction whose nature, severity, consequence or frequency is not consistent with the description of the anticipated risk in the previous protocol or other relevant materials (e.g., investigator's brochure). As the master document, the investigator's brochure provides the reference safety information to judge if one adverse event is expected or unexpected.

3) Serious adverse reaction: defined as an adverse reaction whose seriousness reaches the criteria on serious adverse reaction, including one of the following conditions: death, being life-threatening, permanent or serious disability or loss of function, requiring hospitalization or prolonged hospital stay, as well as congenital anomaly or birth defects after administration of Investigational product.

The sponsor will submit the initial report to the Center of Drug Evaluation of China

Food and Drug Administration, provincial food and drug administration and inform the principal investigator at all the clinical trial institutions in the following time limits based on the nature (category) of SUSAR event.

1) For fatal or life-threatening suspected unexpected serious adverse reaction (SUSAR), the sponsor should report it after awareness of it for the first time as soon as possible and no later than 7 natural days, and report relevant follow-up information within the following 8 natural days.

2) For non-fatal or life-threatening suspected unexpected serious adverse event (SUSAR), the sponsor should report it after awareness of it for the first time as soon as possible and no later than 15 natural days.

3) For other information on the potential serious safety risks, the sponsor should report it after awareness of it for the first time as soon as possible and no later than 15 natural days.

The above is the procedure for SUSAR reporting in China, each study site should report it according to local regulatory requirement.

11.8. Safety reporting

Investigators and the sponsor should provide data on SAE and safety risk evaluation report according to local/national ethics committee and regulatory requirements in time.

11.9. Treatment and management of adverse events

Investigators should establish contingency plan for SAE management in clinical trials, train all the relevant staff, take measures to be aware of any clinically significant disease/event after vaccination, and urge the subjects to go to the designated hospital for appropriate treatment in time. The drugs for treatment of AE should be recorded in the subject's original record and eCRF.

In case of disagreement and dispute in management of adverse events, investigators have the obligation to cooperate with the sponsor to deal with it and assist subjects in medical assessment.

The sponsor has the obligation and responsibility to ensure the safety of subjects unconditionally, and provide humane care and compensation for the subjects with AEs related with the Investigational product during participation in the clinical trial.

For the AEs that are still ongoing at the termination or end of visit, investigators should pay more attention to them continuously, the AEs related with the vaccination should be followed up until they are resolved, and the follow-up of unrelated event, such as disease, can be discontinued upon acquisition of the diagnosis.

11.10. Pregnancy events

All pregnancy events occurred within 1 year from the first dose of vaccine to

the full course of vaccination should be reported within 5 days after being informed and the investigators should fill in the "Pregnancy Report Form".

Investigators will closely follow the pregnant subjects, and obtain information about pregnancy outcomes (for example, details of delivery and newborn situations or termination of pregnancy), and update the "Pregnancy Report Form." The follow up visits for the will last for one year, and whether to continue the follow-up visit will be determined according to the non-clinical results and the one-year observation results.

Pregnancy itself is not considered an SAE, but any complications during pregnancy will be considered as AE and in some cases can be considered as SAE, such as spontaneous abortion, stillborn foetus, stillbirth and congenital abnormalities of infants. When no abnormalities are found in the fetus, induced abortion due to the mother's personal decision is not considered AE.

12. Study Support Teams and Their Responsibilities

12.1. Endpoint Assessment Committee (EAC)

EAC is established to confirm the cases of COVID-19 occurring during study period and judge according to the grading criteria for COVID-19 stipulated in study protocol.

Description of responsibilities:

(1) Able to review, approve and complete related work in a timely manner in accordance with the EAC charter;

(2) The chairman is responsible for supervising whether the review of the endpoint event is carried out in accordance with the trial protocol; shall attend all meetings; record (only all the review results) in the summary report form and sign; responsible for checking meeting minutes and signing; coordinate and reach a consensus, and communicate the views to the sponsor;

(3) In case of any endpoint event, objectively evaluate the endpoint event according to the unified definition standards, combined with clinical expertise and relevant contents in the protocol to determine whether it conforms to the definition standards;

(4) Review the description of all events and check the source documents of each event. Necessary relevant information in the source documents obtained by the committee members have to be masked to ensure that blind review is achieved;

(5) During the independent review process, the committee members can request to provide the required source documents, and review relevant clinical data (i.e.,

lung imaging, death certificate, hospitalization records, etc.) before making the final decision;

(6) Members reach a consensus on the independent review, and the review results will be announced at the meeting and the chairman will sign for confirmation in the summary report form. If the independent review opinions of the committee members cannot reach a consensus, review meetings (regular meetings or ad hoc meetings) will be held as necessary to discuss them. If there are still disagreements after the discussion, voting shall be performed. Voting must also follow certain rules;

(7) If the committee members need additional source documents during the review meeting, they should be recorded in the meeting minutes and make supplementary application after the meeting;

(8) The EAC management team needs to cooperate with the data management department to complete the data question proposal and answer for the review results, which is different from the general clinical trial question answering process;

(9) After completing evaluation, formulate the final Master Binder.

12.2. Data and Safety Monitoring Board (DSMB)

An independent DSMB is established, which is composed of experts possessing the necessary knowledge of clinical trial. Before each meeting, data report is received by DSMB. If the preset conditions are achieved in the study, a formal interim analysis will be made by DSMB. All data reviewed by DSMB are strictly kept secret. In the chapters of DSMB, the responsibility of DSMB, the number of interim reports and the way of operation are stipulated. The interim report is written by independent statisticians. All suggestions of DSMB are conveyed to field PI. Written summary report of DSMB and applicable suggestions are submitted by field PI to local/national ethics committee and other applicable institutions.

Description of responsibilities:

(1) Verify, approve and complete relevant work in time according to the stipulations in chapters of DSMB;

(2) Verify study protocol, verify efficacy/safety data and raise suggestions for revision of monitoring plan;

(3) Execute the verification of data at unblind state according to the monitoring plan. The efficacy/safety data are exhibited at unblind state through the information of

actual study grouping (including: true name of two groups).

(4) If the conditions are allowed, the factors beyond study are explored, such as scientific or therapeutic progress possibly causing a problem in safety of subjects or ethics of study.

(5) Participate in discussion of DSMB, and vote for suggestions of DSMB when necessary;

(6) Suggest the sponsor for other modifications during the course of study and after the termination of study based on the observed data;

(7) If severe, critical or fatal case occurr after the subject is infected with SARS-COV-2 during study period, a special investigation should be conducted. The DSMB shall conduct an analysis based on the findings of the specific investigation. If the analysis suggests that ADE/VED exists, the DSMB shall convene an emergency meeting to assess the risk of ADE/VED in the entire trial and immediately report it to the institutional review board (IRB) / independent ethics committee (IEC) and relevant regulatory authorities.

13. Statistical Analysis

Besides study protocol, an independent statistical analytical plan will be formulated, which illustrates the technical details of statistical analysis in more detailed way. The SAP will be finalized before database lock.

13.1. Research hypothesis

Primary study hypothesis

The lower limit of the 95% confidence interval (CI) for protection against COVID-19 of any severity is greater than 30%, compared with placebo, at least 7 days after full course of immunization.

13.2. Sample size considerations

13.2.1 Sample size calculation based on efficacy study

A large-scale validatory clinical study is made to evaluate the efficacy of Investigational product against COVID-19 in the population of ≥18 years old. By assuming that the occurrence rate of COVID-19 of any severity is 1% during the study period, efficiency of test is calculated. According to the study plan, The trial plan observed 1/3 or 2/3 of cases of COVID-19 of any severity , an interim analysis is made; and total error of type I is controlled within 5% (two-sided) through the consumption function of O'Brien-Fleming. In order to study 60% efficacy of

Investigational product (lower limit of 95% CI is >30%), a total of 156 COVID-19 cases of any severity and 22144 subjects (11072 cases each group) are required to make the power of test reach 90%. The number of events is calculated through exact conditional method according to the large-sample hypothesis of Poisson distribution of Chan and Bohidar. Therefore, by overall considering the conditions of dropout, protocol deviation and incompliance, 14000 subjects will be recruited into each group according to study plan.

13.2.2 Sample size calculation based on immunogenicity bridging study

Set a non-inferior effect value of 0.67, the inspection level α of 0.025 unilateral, and a power of 90%, and assume that the GMT of anti-SARS-COV-2 neutralizing antibody in people in China and outside China is the same, the standard deviation of the antibody titer after logarithmic conversion is 0.55, and the distribution ratio of the two groups of samples is 1:1. Using PASS 15, the minimum sample size of each investigational vaccine group in China and outside China was 207. Considering factors such as shedding and age distribution, 1000 subjects were planned to be enrolled in each experimental vaccine group in China and outside China (750 subjects aged 18-59 and 250 subjects aged 60 and above).Therefore, a total of 2,000 patients are to be enrolled, including 1,000 in China and 1,000 outside China (1,000 subject outside China will be immunogenicity subgroup of the efficacy study cohort, which will also participate in the efficacy evaluation). There will be 1000 in the investigational vaccine group and 1000 in the placebo group.

To sum up, the total sample size of the Phase III clinical trial will be 29,000.

Because of the unpredictable number of cases in some countries and centers and the incidence rate in various countries, the number of COVID-19 cases is checked blindly during the test. The number of subjects planned to enter the group can be increased during the trial according to the incidence rate of different regions, but the number of COVID-19 cases of any severity required can not be changed, so the total type I error will not be inflated.

13.3. Analysis Sets

✧ **Efficacy analysis sets**

Full Analysis Set for Efficacy (E-FAS): It includes all subjects observing the principle for intent-to-treatment (ITT), entering the stage of randomization, completing the inoculation of vaccine for at least one dosing, and receiving at least one follow-up of case monitoring after the inoculation.

Modified Full Analysis Set for Efficacy (E-mFAS): It is a subset of E-FAS; it includes all subjects completing the whole-course inoculation of vaccine and receiving at least one follow-up of case monitoring from 7 days after the whole-course inoculation.

Per-Protocol Set for Efficacy (E-PPS): It includes all subjects conforming to inclusion/exclusion criteria, entering the stage of randomization, completing the whole-course inoculation of vaccine, receiving at least one follow-up of case monitoring from 7 days after the whole-course inoculation and not seriously violating study protocol.

In the E-mFAS and E-PPS, the cases are calculated from 7 days after the whole-course inoculation, which is mainly applied to evaluate primary efficacy of vaccine; in the E-FAS and E-mFAS1, the cases are calculated after the inoculation of vaccine for first dosing.

✧ **Immunogenicity analysis sets**

Full Analysis Set for Immunogenicity (I-FAS) : It includes all subjects observing the principle for intent-to-treatment (ITT), having completed randomization and received at least one dose of vaccine and having valid pre-immunization immunogenicity result.

Per-Protocol Set for Immunogenicity (I-PPS): It includes all subjects conforming to the inclusion and exclusion criteria, having completed full course of vaccination of vaccine and having valid immunogenicity results of both pre-immunization and 14 days after full course of vaccination.

Immune Persistence Set (IPS): It includes all subjects having completed blood sample collection for immune persistence evaluation 6 months after full course of vaccination and having valid antibody data.

I-FAS and I-PPS are applied for immunogenicity analysis and IPS is appiled for immune persistence analysis.

✧ **Safety analysis sets**

Safety Set (SS): It includes all subjects having received at least one dose of Investigational product.

✧ The safety analysis sets will also define the first dose safety analysis set, the second dose safety analysis set and the third dose vaccine safety analysis set. The first dose safety analysis set includes subjects who have completed the first dose of vaccine for post-dose safety analysis; the second dose safety analysis set

includes subjects who have completed the second dose of vaccine for post-dose safety analysis; the third dose safety analysis set includes subjects who have completed the third dose of vaccine for post-dose safety analysis.

In efficacy and immunogenicity analysis, all subjects will be analyzed according to the group they are randomly assigned to; all safety data will be analyzed according to their actual investigational group.

All analysis sets will be discussed by the principal investigator, the sponsor, the statistician and the data manager during a blind data review prior to database locking.

13.4. Statistical analysis method

13.4.1. General principle

Measurement data will be statistically described with mean, median, standard deviation, maximum and minimum; enumeration data or ranked data will be described with frequency and rate.

SAS Version 9.4 or above statistical software will be used for all the statistical analyses.

13.4.2. Study completion and demographic characteristics

The number of subjects screened, enrolled in each group and completing the study, as well as the number of subjects in each analysis set will be summarized, respectively, the drop-out subjects and reasons for drop-out will be analyzed. The demographic characteristics of the subjects in each group will be statistically described.

13.4.3. Evaluation of efficacy

13.4.3.1.Evaluation of primary efficacy

The person-year incidence of any severity of COVID-19 diagnosed and its 95% confidence interval will be calculated in the vaccine group and placebo group 7 days after completion of the full course of vaccination, Poisson regression model is used to carry out statistical comparison of the intergroup difference, the vaccine protection rate and its 95% confidential interval based on person-year incidence will be estimated based on the model. In Poisson regression model, the number of patients is the dependent variable, center, age group (18~59 years, 60 years and above) and group are fixed effects, person-year exposure of subjects is offset, log link function is used. In case the person-year incidence is close to 0 in vaccine group or placebo group, the exact method will be used to calculate the 95% confidential interval of the protection rate based on person-year incidence.

Where, the person-year incidence = (number of patients/person-year exposure of subjects) ×100%. In the calculation of person-year exposure, the start time is 7 days after full course of vaccination, the time of termination of any severity of COVID-19

cases is the time at the first discovery of the case, the time of termination of other subjects is the last follow-up time for observation of efficacy. Vaccine protection rate = 1- (person-year incidence in vaccine group / person-year incidence in placebo group).

The efficacy for any severity of COVID-19 diagnosed 7 days after full course of vaccination will be evaluated based on E-mFAS and PPS.

13.4.3.2 Evaluation of secondary efficacy

In addition, evaluation of the efficacy for severe and critical COVID-19 7 days after full course of vaccination as well as the evaluation of the efficacy of at least one dose of vaccination for any severity of COVID-19 based on FAS use the same statistical analysis method with that for evaluation of primary efficacy.

13.4.4. Immunogenicity evaluation

13.4.4.1. Immunogenicity evaluation in immunogenicity bridging study

The GMT of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group in immunogenicity bridging study is statistically compared in the analysis of covariance model fitted after logarithmic transformation, which uses the GMT of SARS-COV-2 neutralizing antibody following logarithmic transformation 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group as the dependent variable, logarithmic conversion result of SARS-COV-2 neutralizing antibody prior to immunization as covariate, region (including China and outside China) and age group (18~59 years, 60 years and above) as fixed effect, GMT of SARS-COV-2 neutralizing antibody following logarithmic transformation 14 days after full course of vaccination in the pre-immunization negative population as well as the least squares means of intergroup GMT ratio and their 95% confidential interval are calculated for each region based on the model; and upon inverse logarithmic transformation, GMT of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in the investigational vaccine group as well as the least squares means of intergroup GMT ratio and their 95% confidential interval are calculated for each region. The lower limit of two-sided 95% confidential interval of the GMT ratio of SARS-COV-2 neutralizing antibody 14 days after full course of vaccination in the pre-immunization negative population in domestic investigational vaccine group versus oversea investigational vaccine group is calculated, and the immunogenicity will be considered as non-inferior in the investigational vaccine group in China to that outside China if it is >0.67.

The positive rate and positive conversion rate of SARS-COV-2 neutralizing antibody and IgG antibody are calculated in the total population, pre-immunization negative population and pre-immunization positive population at each time point post immunization for different regions (China and outside China), in the investigational

vaccine group in immunogenicity bridging study, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test / Fisher exact probability method is used for the statistical test of the difference across different regions.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI (GMT growth multiple) of SARS-COV-2 neutralizing antibody and IgG antibody 14 days after full course of vaccination in the total population, pre-immunization negative population and pre-immunization positive population for different regions (China and outside China), in the investigational vaccine group in immunogenicity bridging study, respectively, and paired t test following logarithmic transformation is used for statistical testing of the difference across different regions.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and IgG antibody prior to vaccination and 14 days after full course of vaccination is plotted for different regions (including China and outside China).

13.4.4.2. Evaluation of immunogenicity in immunogenicity subgroup

The positive rate and positive conversion rate of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody in the total population, pre-immunization negative population and pre-immunization positive population at each time point post immunization are calculated for vaccine group and placebo group in immunogenicity subgroup, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test / Fisher's exact test probability method is used for statistical test of the difference between vaccine group and placebo group.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody in the total population, pre-immunization negative population and pre-immunization positive population in vaccine group and placebo group in immunogenicity subgroup, respectively, and paired t test following logarithmic transformation is used for statistical testing of the difference across different regions.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody prior to vaccination and 14 days after full course of vaccination is plotted for vaccine group and placebo group in immunogenicity subgroup, respectively.

13.4.4.3. Evaluation of immune persistence

The positive rate of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization is calculated in vaccine group and placebo group in immunogenicity subgroup, respectively, Clopper-Pearson

method is used to calculate its 95% confidential interval, and chi-square test /Fisher exact probability method is used for statistical test of the difference between vaccine group and placebo group.

Geometric mean and two-sided 95% confidential interval are used to statistically describe GMT and GMI of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization in vaccine group and placebo group in immunogenicity subgroup, respectively.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization is plotted for vaccine group and placebo group in immunogenicity subgroup, respectively.

13.4.5. Safety evaluation

MedDRA is used for medical coding of adverse events and serious adverse events, which will be statistically summarized by system organ class (SOC) and preferred term (PT). In addition, the solicited adverse events will be statistically summarized by adverse event at injection site and non-injection site (systemic) as specified in the protocol. Treatment emergent adverse events (TEAE) following vaccination are mainly statistically analyzed in this study, and those occurred prior to vaccination will be presented in a form of list. Unless otherwise noted, the adverse events in the following text are TEAEs.

Number of AEs, number of subjects and incidence of all the adverse events, adverse events related with the Investigational product, adverse events unrelated with the Investigational product, grade 3 and above adverse events, grade 3 and above adverse events related with the Investigational product are calculated in the investigational vaccine group and placebo group, respectively, Fisher exact probability method is used for statistical comparison of the difference in the incidence of the above adverse events between the two groups. The time to occurrence of adverse events, dose time and severity are statistically described.

Adverse events following each dose time of vaccination are statistically analyzed, respectively. Analysis of adverse events following each dose time will be performed on the safety set of each dose time.

List of adverse events related and unrelated with the Investigational product will be presented.

Number of AEs, number of subjects and incidence of all the serious adverse events, serious adverse events related with the Investigational product and serious adverse events unrelated with the Investigational product are calculated in investigational vaccine group and placebo group, respectively, Fisher exact probability method is used for statistical comparison of the difference in the incidence of the above adverse events between the two groups. List of serious adverse events will be presented.

13.5. Interim analysis (IA)

This is a case-driven study. If 52 cases of COVID-19 (1/3), 104 cases of COVID-19(2/3) are observed during the study course, interim analysis will be made. Total error of type I is controlled through the consumption function of O'Brien-Fleming. When 52 cases of COVID-19 of any severity are observed and the first interim analysis is made, nominalα for IA is $\alpha_1 = 0.0001$ (single-sided) as calculated through the consumption function of O'Brien-Fleming; If no valid conclusion is obtained in the first interim analysis, the second interim analysis will be performed when 104 cases of COVID-19 are collected. The nominal test level of this interim analysis is $\alpha_2 = 0.0060$ (unilateral); if no effective conclusion is reached in the second interim analysis, the final analysis will be performed when 156 cases of COVID-19 are observed . Nominal test level for FA is $\alpha_2 = 0.0231$ (single-sided). During the study course, nominal significance level of IA and FA is estimated through the consumption function of O'Brien-Fleming according to the actual number of observed cases of COVID-19 at IA. If there are more than planned cases of COVID-19 at IA, α is assigned again through the method ofO'Brien-Fleming.

13.6. Multiplicity

The O'Brien Fleming consumption function is used to control the total type I errors within 5% during the interim analysis. Please refer to the interim analysis section for details.

13.7. Statistical analysis strategy

This study will be able to conduct statistical analysis for NMPA declaration when the above sufficient number of events is reached; after 1-year observation and follow-up, the protective effect of vaccine will be further evaluated based on all collected cases.

14. Data management

14.1. eCRF design

eCRF will be designed in accordance with the study procedures and flow chart in the protocol, needs to be reviewed jointly by project manager, data administrator, statistician and protocol writer after formation of the draft, meet the protocol and comply with relevant laws and regulations, and the process of version control needs to be completely recorded.

14.2. Guideline on eCRF filling

The guideline on eCRF filling is the detailed description on filling in each page and each data point of eCRF according to the study protocol. Acquisition of eCRF and guideline on its filling at the clinical trial center needs to be guaranteed prior to enrollment of subject, and relevant staff at the clinical trial center will be trained on the protocol, eCRF filling and data submission process, which needs to be archived for record.

14.3. Note to eCRF

Note to eCRF is the marking to blank eCRF, record of the place of each data item in eCRF as well as the variable name and code in the database. All the data items in eCRF need to be marked. DM review is required.

14.4. Database design

The database should be established in accordance with the name of dataset, name of variable, type and length of variable in noted eCRF, and comply with the structure and configuration of standard database as much as possible. After completion of establishment of database, the database should be tested, the database test report needs to be issued and signed by the person in charge of data management for confirmation.

14.5. Permission assignment

According to different roles, accounts will be created by system administrator separately, and different permissions will be granted.

14.6. eCRF filling

The study personnel need to collect subject’s data according to the requirements in the study protocol, and fill the data in eCRF in an accurate, timely, complete and standard manner according to the guideline on filling, based on the original materials. Modification of the data on eCRF must comply with the standard operating procedures, and the modification traces need to be maintained.

14.7. Transmission and resolution of questions

Data Management (DM) will list a detailed data verification plan, which will be signed by data administrator, data manager and the sponsor for confirmation upon review and no objection by the sponsor, medical staff, statistician and project manager. After entry of data in EDC, the system will verify the data in accordance with the Edit Check established in the data verification plan and send queries automatically for all the questionable data; a manual query will be sent through EDC for the data for which the system can not be set to send query, the entry clerk or investigator will confirm and answer manual queries and system queries, and modify the wrong data when necessary, until the query is solved. If the answer can not solve query, a query can be made on this data point again by data administrator and clinical monitor, all the traces will be kept in EDC database.

14.8. Data modification and review

After the data are verified by data-entry clerk or investigator, the data can be modified, the modified data need to be prompted in the system and the reason for modification should be shown in the system. Investigators can finally verify all data.

14.9. Medical coding

Adverse events collected in clinical trials will be encoded using standard dictionary. MedDRA is the standard dictionary commonly used. The dictionary and version used for coding should be clearly recorded for the data set coded.

14.10. Comparison of SAE consistency

All the data points related with SAE in the database will be compared with the data points in PV (Pharmacovigilance) system using program, inconsistent data need to be communicated with PV personnel, until no difference in the data.

14.11. Data review meeting

Before locking of database, the draft of data review report and all the data lists will be well prepared, the database will be finally reviewed by the sponsor, investigators, data administrator and statisticians together, and division of statistical analysis population, verification of serious adverse event report and treatment record, verification of ADE/VED report and treatment record will be carried out according to the clinical study protocol, the data review report and population division plan need to be finalized after the data review meeting.

14.12. Lock and unlock of database

Lock of database is one important milestone during clinical study. The process and time of locking should be clearly documented, locking is the cancellation of the permission to edit the database, any unauthorized account can not manipulate the database. If there is any modification after lock of database, it needs to be applied and can be executed only after discussion and signature by the sponsor, investigators, statisticians, clinical monitors and data administrator for confirmation, and the reason for unlocking needs to be recorded carefully.

15. Maintenance and Management of Material

15.1. Management of original material

The informed consent form, vaccination and follow-up record book, diary card, contact card, SAE report form and other original materials are important basis for traceability of clinical trials, should be recorded in a timely, accurate, complete, standard and authentic manner, and properly maintained at the study site.

The study data will be entered in EDC by authorized and specially trained investigators in accordance with the original materials, can not be changed ad

arbitrium during the entry, and should be modified in accordance with the guideline on filling for wrong entry. In order to ensure the authenticity and reliability of the clinical trial data, EDC will be reviewed by monitors and investigators jointly. All the materials will be statistically processed by the unit in charge of the clinical trial or the statistician entrusted by the sponsor upon signature of investigators.

15.2. Study material

The sponsor and study party will provide clinical trial materials in accordance with the regulations on drug registration management and GCP provisions.

Investigator's folder will be arranged as required by GCP and maintained at the study site. The study site is responsible for arranging and summary of the materials delivered to the sponsor. Materials recording true information of subjects, such as screening registration form, informed consent form, vaccination and follow-up record book, diary card, contact card, subject’s medical record, will be sealed at the study site, the coordinators in charge of the institutions will check and hand them over with the on-site archivist, both parties will sign the deposit agreement or memorandum.

File management will be carried out according to SOP, the identification label, including name of project, date of completion, sponsor and storage period, will be well prepared, insect-proof, moisture-proof, fire-proof and anti-theft measures will be taken. Use and access to the materials of this project is only limited to relevant staff in the project, the personnel from the sponsor (including clinical monitors) and inspectors from regulatory authorities. All the materials for application of drug registration will be maintained until 5 years after approval of the vaccine, the sponsor will be informed on the due date, the materials can not be disposed by anyone without authorization, prior to acquisition of the written notification from the sponsor.

16. Quality Control and Quality Assurance Procedures

This study will be carried out in accordance with relevant regulations and standard operating procedures.

The study will be conducted in accordance with this protocol, the ICH GCP) and any applicable regulatory requirements. Biological samples will be processed, stored and transported in accordance with SOP.

Data validation will be performed to identify errors or discrepancies to ensure the integrity, validity and accuracy of the data.

17. Ethical and Legal Considerations

17.1 Declaration of Helsinki

The investigators assure that this study will be carried out in accordance with the principles from Declaration of Helsinki.

17.2 Good Clinical Practice

The investigators assure that this study will be performed in compliance with the Good Clinical Practice.

17.3 Review of the clinical trial

The ethics committee shall review the scientific and ethical rationality of the drug clinical trial project in order to ensure the dignity, safety and rights of the subjects, promote the scientific and healthy development of the drug clinical trial, and enhance the public's trust and support for the drug clinical trial.

The ethnics committee may review the test protocol, informed consent, recruitment materials and other written materials provided to the subjects. The revision of the protocol shall be negotiated with the sponsors. The content of the informed consent form may be accepted by the ethnics committee if it does not violate the protocol and conforms to the local actual conditions.

17.4 Confidentiality of subject information

The study teams will keep the confidentiality of subject information. In CRF, initials of name of subjects can be indicated; in other study documents and electronic database, the identity of subjects can be indicated only through the study No. of subjects. All documents are stored in safe way, and can be accessed only by study team and authorized persons.

17.5 Compensation

The subjects will not be paid for taking part in this study. The expenses of subjects incurred during study period is reimbursed according to the local applicable guidelines and the policies of ethics committee.

17.6 Report

From the approval date of study, annual progress report is annually submitted by PI to all applicable ethics committees. In addition, after the completion of study, a study termination report is submitted by PI to all applicable ethics committees.

18. Insurance

An insurance of clinical trial is arranged for subjects. If a harm related to clinical trial occurs in the subjects during the course of clinical trial, the corresponding compensation will be made.

19. Paper Publishing

After the conclusion of the trial, the research unit may publish the summary

report or research results involving the clinical trial in the form of a paper after obtaining the written authorization of the sponsor, and the researchers of the research unit and the technical cooperation unit (pharmacodynamic evaluation) shall have the right of authorship of the paper. Negative or inconclusive research results should be published or made public in the same way as positive results.

20. References

1. Novel Coronavirus Infection Diagnosis and Treatment Plan (trial version 7), China National Health Commission, March 3rd, 2020.
2. Zumla A, Chan JFW, Azhar EI, et al. Coronaviruses-drug discovery and therapeutic options［J］. Nat Rev Drug Discov, 2016,15(5):327-347．.
3. Graham RL, Donaldson EF, Baric RS. A decade after SARS:strategies for controlling emerging Coronaviruses［J］. Nat RevMicrobiol, 2013, 11(12):836-848.
4. Li W, Moore MJ, Natalya Vasilieva, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS Coronavirus ［J］.Nature, 2003, 426(6965):450-454
5. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human Coronavirus-EMC［J］.Nature, 2013, 495(7440):251-254.
6. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 CoronavirusNovel Coronavirus: implications for virus origins and receptor binding［J］. Lancet, 2020. DOI: 10.1016/S0140-6736(20)30251-8
7. Guideline on the Classification of Adverse Events in Clinical Trials of Preventive Vaccines, NMPA, December 31, 2019
8. Good Clinical Practice of Pharmaceutical Products (GCP), NMPA, April 23, 2020.
9. ICH E6, GCP, 1996

21. Annex A: Subject Study Workflow

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
Time of visit (days)	**D-7~0**	**D0**	**V2+8**	**V2+30**	**V4+8**	**V4+30**	**V6+8**	**V6+14**	**V6+30**	**V6+180**	**V6+360**
Window Period(days)	/	/	/	**7**	/	**7**			/	**30**	/
Informed Consent	X										
Demographic information	X										
Medical history and allergy history	X										
Recent medication (vaccine) history	X										

Vital signs (axillary / oral temperature, blood pressure, pulse)		X									
Physical examination	X										
Axillary temperature /Oral temperature				X		X					
SARS-CoV-2 RT-PCR	X										
SARS-CoV-2 IgG and IgM	X										
Urine pregnancy		X									
Verification of inclusion/exclusion	X	X									

criteria											
Randomization		X									
Vaccination		X		X		X					
Observation of 30 minutes after vaccination		X		X		X					
Dispensing the thermometers, dipperstick, diary cards and implementing the trainings 3		X		X		X					

Retrieving diary cards, and distribution of contact cards			X		X		X				
Retrieving contact cards				X		X			X		
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Blood sampling for immunological assay1		X						X		X	
Report serious adverse events		X	X	X	X	X	X	X	X	X	X
Report pregnancy event		X	X	X	X	X	X	X	X	X	X

Monitoring of COVID-19 cases2		X	X	X	X	X	X	X	X	X	X

1. Immunological blood samples were collected only for subjects of immunogenicity subgroup.

2. If suspected cases of COVID-19 are found after the inoculation for the first dosing, nasopharyngeal swab is collected for RT-PCR test.

3. At Visit 2, the thermometer, straight ruler and diary card is granted; at Visit 4 and Visit 6 the diary card is granted.

4. Only the concomitant medications for treating SAE and pregnant complications are collected from 30 days to 12 months after the whole-course inoculation of vaccine.

22. Annex B: Adverse Events Grading Scale

Clinical observation indicators (Table 1-3)

Table 1. Injection-site (local) Adverse Events Grading Scale

Symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Pain and tenderness (optional; and tenderness is applicable for the subjects unable to express the pain by themselves)				
Pain	No or slight influence on the activity of limbs	Influencing the limb movement	Influencing the daily life	Loss of basic ability for self care, or causing a hospitalization
Tenderness	Resistance and withdrawal at a contact or touch	Comfortable crying at a contact or touch	Uncomfortable continuous crying	Requiring the emergency care or hospitalization
Induration*, swelling (optional) ** #				
> 14 years old	Diameter 2.5~or area 6.25~2 and with no or slight influence on daily life	Diameter 5~ or area 25~2 or influence on daily life	Diameter ≥10 cm or area ≥100 cm2or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious influence on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue
Rash*, redness (optional) ** #				
> 14 years old	Diameter 2.5~5 or area 6.25~2 and with no or slight influence on daily life	Diameter 5~ or area 25~2 or with an influence on daily life	Diameter ≥10 cm or area ≥100 cm cm cm2 or ulceration or secondary infection or phlebitis or aseptic abscess or wound drainage or serious influence on daily life	Abscess, exfoliative dermatitis, necrosis of dermis or deep tissue

Symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Others				
Pruritus	Pruritus at the site of inoculation, which is relieved spontaneously or within 48 hours after the treatment.	Pruritus at the site of inoculation, which is not relieved within 48 hours after the treatment.	Influencing the daily life	NA
Cellulitis	NA	Requiring the non-injection treatment (e.g., oral administration of antibiotic, antifungal, and antiviral drugs)	Required treatment of intravenous injection (such as intravenous injection of antibacterial, antifungal and antiviral drugs)	Pyemia/sepsis, or tissue necrosis

Note: *The diameter is directly measured for grading evaluation, and the progress/change of measurement results is also recorded.

**Maximum measured diameter or area is adopted.

The induration, swelling, rash and redness are evaluated and graded according to the functional grade and the actual measurement results through the indices of higher grade.

Table 2. Vital Signs Grading Scale

Signs	Grade 1	Grade 2	Grade 3	Grade 4
Fever* [axillary temperature (°C)]				
> 14 years old	37.3~<38.0	38.0~<38.5	38.5~<39.5	≥39.5, lasting for over 3 days
Electrocardiogram PR interval prolonged or atrioventricular block (optional)				
> 16 years old	PR interval: 0.21s - <0.25s	PR interval ≥0.25 seconds or type I atrioventricular block of Degree 2	Type II atrioventricular block of Degree 2 or ventricular interval ≥3 seconds	Complete atrioventricular block
Heart rate				
Tachycardia (times/min)	101~115	116~130	>130	Arrhythmia requiring an emergency treatment or a hospitalization
Bradycardia (times/min)	50~54	45~49	<45	Arrhythmia requiring an emergency treatment or a hospitalization
Blood pressure				
Hypertension (mmHg)				
≥18 years old	Systolic pressure 140~or diastolic pressure 90~<100	Systolic pressure ≥160~or diastolic pressure ≥100~<110	Systolic pressure ≥180or diastolic pressure ≥110	Occurrence of life-jeopardizing complications not diagnosed previously (such as malignant hypertension) or causing a hospitalization
Hypotension (systolic blood pressure) (mmHg)	85~<89	80~<85	<80	Shock or causing a hospitalization
Respiratory frequency (times/min)	17~20	21~25	>25	Requiring tracheal intubation

Note: * In China, axillary temperature is generally adopted, which is converted into oral temperature and anal temperature when necessary. Generally, the formula for conversion is adopted: Oral temperature = Axillary temperature + 0.2°C; Anal temperature = Axillary temperature + (0.3~0.5°C). When a continuous high fever

occurs, the reason for high fever should be determined as soon as possible.

Table 3: Grade of adverse events in whole body (except the injection site)

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal system				
Diarrhea	Mild or transient, 3~4 times a day, abnormal property of stool, or mild diarrhea continuously for <1 week	Moderate or continuous, 5~7 times a day, abnormal property of stool, or diarrhea continuously for >1 weeks	>7 times a day, abnormal property of stool, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, necessary for intravenous infusion at >2 L	Hypotensive shock, requiring hospitalization
Constipation*	Necessary for stool softeners and diet adjustment	Necessary for laxatives	Refractory constipation necessary for manual defecation or enema application	Toxic megacolon or intestinal obstruction
Swallowing difficult	Mild discomfort when swallowing	Dietary restrictions	Very limited in diet and talk; unable to take solid food	Unable to take liquid food; necessary for intravenous infusion of nutrients
Anorexia	Decreased appetite but no reduction of food intake.	Inappetence, decrease of food intake, no obvious decrease of body weight	Decreased appetite with significant weight loss	Requiring the intervention measures (e.g., gastric tube feeding, and parenteral nutrition)
Vomiting	1-2 times per 24 hours and no influence on movements	3-5 times per 24 hours or limited movements	>6 times within 24 hours or necessary for intravenous infusion	Necessary for hospitalization or nutritional support through other channels due to the hypotensive shock

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	Transient (or intermittent and basically normal intake of food	Continuous nausea causing a decrease of food intake (24~48 hours)	Continuous nausea causing a hardly intake of food (>48 hours) or necessary for intravenous fluid infusion	Life-threatening (such as hypotensive shock)
Musculoskeletal and connective tissues disorders				
Pain muscle (non-injection site)	Not influencing the daily life	Slightly influencing the daily life	Serious muscular pain with a serious influence daily life.	Emergency care or hospitalization
Arthritis	Mild pain with inflammation, erythema, or joint swelling; not limiting the functions	Moderate pain with inflammation, erythema, or joint swelling; limiting the functions, but not influencing the daily life	Severe pain with inflammation, erythema or joint swelling; influencing the daily life	Permanent and/or disabling injury of joint
Pain joint	Mild pain, but not limiting the functions	Moderate pain; necessary for analgesics and/or causing a dysfunction, but not influencing the daily life	Serious pain; necessary for analgesics and/or influencing the daily life	Disability pain
Nervous system				
Headache	Not influencing the daily life, requiring no treatment	Transient, slightly influencing the daily life, and possibly requiring a treatment or intervention	Serious influence on daily life, requiring a treatment or intervention	Refractory, requiring an emergency treatment or a hospitalization
Syncope	Near-syncope without unconsciousness (such as pre-syncope)	Unconsciousness, but unnecessary for treatment	Loss of consciousness, requiring treatment or hospitalization	NA
Newly occurring convulsions				

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
≥18 years old	NA	NA	Convulsions with 1-3 times	Convulsion for a long time and for several times (such as status convulsivus) or at uncontrollable state (such as refractory epilepsy)
Respiratory system				
Cough	Transient, requiring no treatment	Constant coughing, with effective treatment	Paroxysmal cough uncontrollable through the treatment	Emergency care or hospitalization
Acute bronchospasm	Transient; unnecessary for treatment; FEV1% 70%~80%	Necessary for treatment; resolvable through the bronchodilators; FEV1% 50%~70%	Unresolvable through the bronchodilators; FEV1% 25%~50% or continuous sinking of intercostal area	Cyanosis; FEV1% <25%; or necessary for intubation
Dyspnea	Exercise dyspnoea	Dyspnea in normal activities	Dyspnea when rest	Dyspnea, necessary for oxygen inhalation, hospitalization or assisted respiration
Skin and subcutaneous tissue disorders				
Non-injection site pruritus (no skin injury)	Mild pruritus, with no or slight influence on daily life	Pruritus, influencing the daily life	Pruritus causing unable activity of daily life	NA
Skin and mucosa abnormality	Erythema/pruritus/color changed	Diffusive rash, maculopapule, dry skin, desquamation	Herpes, exudation, desquamation /ulcer	Exfoliative dermatitis involving mucosa, or erythema multiforme, or suspected Stevens-Johnsons syndrome
Nervous system				
Insomnia*	Mild insomnia, with no or slight influence on daily life.	Moderate sleeping difficulty, influencing the daily life	Serious insomnia, seriously influencing the daily life, requiring a treatment or hospitalization	NA

Organ system symptoms/signs	Grade 1	Grade 2	Grade 3	Grade 4
Irritation or suppression	Mild irritation or slight suppression	Irritability or somnolence	Uncomfortable or low response	NA
Mental disorder (including: anxiety, depression, mania and delirium) Symptoms should be reported in detail	Mild symptoms, unnecessary for hospital visit or behavior with no or slight influence on daily life.	With clinical symptoms, necessary for hospital visit or behavior influencing the daily life.	Necessary for hospital visit or behavior ability not supporting the daily life.	Tendency to injure the self or others or acute delirium or loss of basic ability for self care
Immune system				
Acute allergic reaction**	Localized urticaria (vesicle), unnecessary for treatment	Localized urticaria necessary for treatment or mild angioedema unnecessary for treatment	Extensive urticarial or angioedema necessary for treatment or mild bronchospasm	Allergic shock or life-jeopardizing bronchospasm or throat edema
Others				
Fatigue, asthenia	Not influencing the daily life	Influencing the daily life	Serious influence on daily life, unable to work	Emergency care or hospitalization
Non-injection site pain# (please note the site when reporting)	Mild pain, not or slightly influencing the daily life	Pain, influencing the daily life	Pain, with a failure in the daily life	Injuring/disabling pain, loss of basic ability for self care

Note: FEV1%: Forced expiratory volume in one second; (FEV1) / Forced vital capacity (FVC)

*: In the subjects with constipation and insomnia, an attention should be paid to the change before and after the inoculation.

**Type I hypersensitivity

#: Pains other than the pain at the site of inoculation (except muscular pain, joint pain and headache).

General Principles for the Grading of Other Adverse Events

The severity of adverse events not listed in the grading scale is evaluated according to the following criteria:

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild: for a short time (<48 hours) or mild discomfort, not influencing the daily life, unnecessary for treatment.	Moderate: Slight or middle restraint of activity, possibly requiring a hospital visit, unnecessary for treatment or only requiring a slight treatment.	Severe: significant movement limitation Restricted, necessary for hospital visit and treatment, possibly requiring a hospitalization	Critical: Possibly life-jeopardizing, serious restraint of activity, necessary for monitored treatment.	Death

REFERENCES

1. NMPA Guideline on the Classification of Adverse events in Clinical Trials of Preventive Vaccines. December 31, 2019

23.Annex C: COVID-19 Case Clinical Material

The information to be collected includes but not limited to RR, blood gas analysis (SpO2, PaO2)/ FiO2), imaging examination results (chest X-ray or chest CT), laboratory examination results (blood routine, liver and kidney function, coagulation analysis, C-reactive protein), respiratory failure (mask oxygen inhalation, nasal high flow oxygen therapy, noninvasive ventilation, mechanical ventilation, ECMO), shock, ICU admission, sepsis, acute pulmonary embolism, acute coronary syndrome, and acute stroke combined delirium, other organ failure, death, etc.

For the above examinations and tests, the original test sheet should be copied as much as possible, and the inpatients' records should be copied.

Annex 2：

Revision History of Phase Ⅲ Clinical Trial Protocol of Recombinant Novel Coronavirus Vaccine (CHO Cell) (ZF2001)

Project Title: A randomized, double-blind, placebo-controlled phase III clinical trial in subjects aged 18 and above inoculated with Recombinant Novel Coronavirus Vaccine (CHO Cell) (ZF2001) to evaluate the efficacy and safety of ZF2001 in COVID-19 prevention

Protocol No.: LKM-2020-NCV-GJ01

I. Description of protocol revision (revised V1.0 into V1.1)

No.	Before Revision（V1.0，2020 11.06）	After Revision（V1.1，2021 02.18）	Revision description
1	Study Team Sponsor's contact address: No.93 Fushan Road, Hefei High Tech Industrial Development Zone, Anhui Province	No.93 Kexue Avenue, Hefei High Tech Industrial Development Zone, Anhui Province	Correct the address information
2	Suspected COVID-19: Those who meet any of the following conditions: (1) Fever (axillary temperature ≥37.3 ℃ / oral temperature ≥37.5℃) combined with cough or fever combined with expectoration or fever combined with shortness of breath; (2) having two or more of the following acute signs or symptoms for 48 hours or above: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell/taste .	Suspected COVID-19: Those who meet any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia/nausea/vomiting, loss of smell/taste .	Revise the definition of suspected case of COVID-19.
3	COVID-19: The result of Real-time fluorescence quantitative RT-PCR test for SARS-COV-2 nucleic acids was positive twice and having any of the following two conditions: (1) Fever	COVID-19: Suspected cases were sampled twice (two samples were collected for each time, one for testing and one for backup storage) , at a sampling interval of 24-48h. The result of	Revise the definition of COVID-19 case.

	(axillary temperature ≥37.3 ℃ / oral temperature ≥37.5 ℃) combined with cough or fever combined with expectoration or fever combined with shortness of breath; (2) having two or more of the following acute signs or symptoms for 48 hours or above: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia, nausea, vomiting, loss of smell/taste.	Real-time fluorescence quantitative RT-PCR test for SARS-COV-2 nucleic acids was positive at least once and having any of the following conditions: fever, cough, expectoration, shortness of breath, chills, fatigue, myalgia, sore throat, stuffiness, headache, diarrhea, anorexia/nausea/vomiting, loss of smell/taste.	
4	**7.2.1 Diagnosis procedure of suspected cases** 1) If the result of RT-PCR test is positive, sampling after 24 hours for re-test should be conducted (If it is not possible to re-sample the subject due to isolation or other disposal measures as required by local epidemic prevention and control policies, a second nasopharyngeal swab previously collected will be used for re-testing) for the respiratory rate (RR), oxygen saturation (SpO2) and oxygenation index (PaO2/FiO2) ① If the result of the re-test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be managed according to the local epidemic diagnosis and treatment measures. ②If the result of the re-test is negative, the case shall be determined as a non-COVID-19 case.	**7.2.1 Diagnosis procedure of suspected cases** 1) If the result of RT-PCR test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be managed according to the local epidemic diagnosis and treatment measures, and samples were taken again for testing within 24-48 hours. 2)If the result of RT-PCR test is negative, samples were taken again for testing within 24-48 hours, and the testing of respiratory rate (RR), oxygen saturation (SpO2) and oxygenation index (PaO2/FiO2) was performed. ①If the result of re-sampled RT-PCR test is positive, the investigators shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects.	Make corresponding revisions according to the revised contents of items 2 and 3; add descriptions about the types of nucleic acid monitoring specimens.

	2).If the result of RT-PCR test is negative, the investigators will conduct follow-up visits to the subjects. If the symptoms continue or the original symptoms worsen or new relevent symptoms appear within 2 days, re-sample(at least 48 hours interval with the previous nucleic acid detection time) and the real-time fluorescent quantitative RT-PCR detection will be performed again . ①If the result of re-sampled RT-PCR test is positive, the procedures from a. shall be carried out. ②If the result of re-sampled RT-PCR test is negative, it will be determined as a non-COVID-19 case. 3）If the result of RT-PCR test is negative, and the symptoms of the subjects disappear or alleviate or no new related symptoms are found within 2 days of during the follow-up visits, the follow-up visits can be stopped and the case can be determined as a non COVID-19 case.	②If the result of re-sampled RT-PCR test is negative, the investigators will follow up the subjects. If the original symptoms continue or worsen or new related symptoms of suspected cases appear within 2 days, the subjects will be monitored according to the content specified in "(1)" and sampled again for testing. The follow-up can be discontinued if the original symptoms of the subjects disappear and no new related symptoms of suspected cases occur during the follow-up. For patients with suspected COVID-19, especially with severe illness, a single negative upper respiratory tract (URT) sample does not exclude the diagnosis, and additional URT and lower respiratory tract (LRT) samples (sputum, endotracheal aspirate, or alveolar lavage) are recommended. LRT (vs URT) samples are more likely to be positive and last longger. The investigators could elect to collect only LRT samples when these are readily available (for example, in mechanically ventilated patients).	
5	7.2.2 Determination of COVID-19 Severity if not hospitalized, subjects shall be treated according to the local epidemic diagnosis and treatment measures, and home care should be taken to take personal and family epidemic prevention measures, followed up by investigators through face-to-face interview, telephone, text message or network everyday, as to collect the data on the course of disease, until its outcome and record in the case surveillance form. If the subject has difficulty breathing during the follow-up, the investigator should remind the	7.2.2 Determination of Covid-19 Severity if not hospitalized, subjects shall be treated according to the local epidemic diagnosis and treatment measures, and home care should be taken to take personal and family epidemic prevention measures, followed up by investigators through face-to-face interview, telephone, text message or network everyday, as to collect the data on the course of disease (including start time and end time of each symptom, whether new symptoms, etc.) , until its outcome and record in the case surveillance form. If the	Further refine the disease course information to be collected.

	subject to go to the hospital immediately for treatment	subject has difficulty breathing during the follow-up, the investigator should remind the subject to go to the hospital immediately for treatment	
6	7.2.3.1 COVID-19 Case re-confirmation The investigators shall collect clinical data from all subjects who are positive for both PCR tests and submit the data to the Endpoint Determination Committee (EAC), which will review the COVID-19 case in accordance with the procedure set forth in the EAC's statutes. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.	7.2.3.1 COVID-19 Case re-confirmation The investigators shall collect clinical data from all subjects who are positive for PCR tests and submit the data to the Endpoint Determination Committee (EAC), which will review the COVID-19 case in accordance with the procedure set forth in the EAC's statutes. At the same time, under the premise of conforming to the policies of target countries, an independent third party can be invited to review the positive samples in response to review requirements. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.	Make corresponding revisions according to the revised contents of items 2 and 3; add description of third-party review.

II. Description of protocol revision (revised V1.1 into V1.2)

No.	Before Revision（V1.1，2021 02.18）	After Revision（V1.2，2021 05.12 日）	Revision description
7.2.1 Diagnosis procedure of suspected cases			

1	2. Investigators will confirm if the definition of suspected case is met based on the symptoms provided by subjects and fill in case surveillance form. (1) If yes, subjects will arrive at the study institutions or designated medical institutions as early as possible for the following examinations, as instructed by investigators. Two samples of nasopharynx swabs will be collected by on-site investigators(two samples will be collected for each sampling). One of the samples should be detected by real-time fluorescent quantitative RT-PCR, and the other should be kept as a backup. Respiratory rate (RR), oxygen saturation (SpO2), oxygenation index (PaO2 / FiO2) will be performed. The investigators will collect the RT-PCR test reports, diagnosis source file and related inspection report sheet of all subjects with positive results in PCR tests: (1) If the result of RT-PCR test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be managed according to the local epidemic diagnosis and treatment measures, and samples were taken again for testing within 24-48 hours.	2. Investigators will confirm if the definition of suspected case is met based on the symptoms provided by subjects and fill in case surveillance form. (1) If yes, subjects will arrive at the study institutions or designated medical institutions as early as possible for the following examinations, as instructed by investigators. Two samples of nasopharynx swabs will be collected by on-site investigators(two samples will be collected for each sampling). One of the samples should be detected by real-time fluorescent quantitative RT-PCR, and the other should be kept as a backup. Respiratory rate (RR), oxygen saturation (SpO2), oxygenation index (PaO2 / FiO2) will be performed. The investigators will collect the RT-PCR test reports, diagnosis source file, mutations of S gene test report and related inspection report sheet of all subjects with positive results in PCR tests: (1) If the result of RT-PCR test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be managed according to the local epidemic diagnosis and treatment measures, and samples were taken again for testing within 24-48 hours. Meanwhile, ARMS-PCR should be employed to detect	Several remarkable SARS-CoV-2 mutants have emerged in the last few months, such as the B.1.1.7 mutant in the United Kingdom, the P.1 mutant in Brazil, and the B.1.351 mutant in South Africa. The important mutations include N501Y, A570D, HV69-70del, K417N, K417T, E484K, etc. In this study protocol, testing for S gene mutation site of Novel Coronavirus is added for subjects with positive PCR, so as to accumulate more comprehensive test data.

		the mutations of S gene of novel Coronavirus, mainly including N501Y, A570D, HV69-70del, K417N, K417T and E484K..	
2	2) If the result of RT-PCR test is negative, samples were taken again for testing within 24-48 hours, and the testing of respiratory rate (RR), oxygen saturation (SpO2) and oxygenation index (PaO2/FiO2) was performed. ①If the result of re-sampled RT-PCR test is positive, the investigators shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects.	2). If the result of RT-PCR test is negative, samples were taken again for testing within 24-48 hours, and the testing of respiratory rate (RR), oxygen saturation (SpO2) and oxygenation index (PaO2/FiO2) was performed. ①If the result of re-sampled RT-PCR test is positive, the investigators shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. Meanwhile, the mutations of S gene of novel Coronavirus should be detected..	

7.4 Biological Sample Collection			
3	2）The biological sample collected at each site will be divided in duplicate, one for real-time fluorescence quantitative RT-PCR at the laboratory for detection of novel Coronavirus nucleic acid (based on the SOP for detection of novel Coronavirus nucleic acid); the other one archived and stored in qualified laboratory for back-up.	2）The biological sample collected at each site will be divided in duplicate, one for real-time fluorescence quantitative RT-PCR and/or detection of mutations of S gene of novel Coronavirus at the laboratory for detection of novel Coronavirus nucleic acid (based on the SOP for detection of novel Coronavirus nucleic acid and the SOP for detection of mutations of S gene of novel Coronavirus); the other one archived and stored in qualified laboratory for back-up.	Make the corresponding revisions according to the revised contents of item 1

III. Description of protocol revision (revised V1.2 into V1.3)

No.	Content before revision (V1.2, May 12, 2021)	Content after revision (V1.3, June 12, 2021)	Revision description
1 General Information			
1	**Study design** Effectiveness evaluation: To evaluate, 14 days after the full course of vaccination, the	**Study design** Effectiveness evaluation: To evaluate, 7 days after the full course of vaccination, the	The evaluation time point of the protective efficacy

	morbidity of and the protective efficacy against COVID-19 of any severity.	morbidity of and the protective efficacy against COVID-19 of any severity.	study endpoints was revised from "14 days after the full course of vaccination" to "7 days after the full course of vaccination"
2	**Study endpoint** Primary study endpoints: (1) Protective efficacy study endpoint: The number of COVID-19 cases of any severity 14 days after the full vaccination.	**Study endpoint** Primary study endpoints: (1) Protective efficacy study endpoint: The number of COVID-19 cases of any severity 7 days after the full vaccination.	
	Secondary study endpoints: (1) Protective efficacy study endpoint: ①The number of severe or critical COVID-19 cases 14 days after the full vaccination; ②The number of COVID-19 cases of any severity after the first dose of vaccination; ③The number of COVID-19 cases of any severity in different age groups (18-59 years old, 60 years old and above) 14 days after the full vaccination.	Secondary study endpoints: (1) Protective efficacy study endpoint: ①The number of severe or critical COVID-19 cases 7 days after the full vaccination; ②The number of COVID-19 cases of any severity after the first dose of vaccination; ③The number of COVID-19 cases of any severity in different age groups (18-59 years old, 60 years old and above) 7 days after the full vaccination.	
3	**Study hypothesis** Main research hypothesis: At least 14 days after the full course of immunization, the lower limit of the 95% confidence interval (CI) of the vaccine effectiveness to prevent COVID-19 of any severity is greater than 30% compared with placebo.	**Study hypothesis** Main research hypothesis: At least 7 days after the full course of immunization, the lower limit of the 95% confidence interval (CI) of the vaccine effectiveness to prevent COVID-19 of any severity is greater than 30% compared with placebo.	
5.2 Study endpoint			
4	**5.2.1 Primary study endpoints:** (1) Protective efficacy study endpoint: The number of COVID-19 cases of any severity 14 days after the full vaccination.	**5.2.1 Primary study endpoints:** (1) Protective efficacy study endpoint: The number of COVID-19 cases of any severity 7 days after the full vaccination.	Same revision description as above

	5.2.2 Secondary study endpoints: (1) Protective efficacy study endpoint: 1) The number of severe or critical COVID-19 cases 14 days after the full vaccination; 2) The number of COVID-19 cases of any severity after the first dose of vaccination; 3) The number of COVID-19 cases of any severity in different age groups (18-59 years old, 60 years old and above) 14 days after the full vaccination.	**5.2.2 Secondary study endpoints:** (1) Protective efficacy study endpoint: 1) The number of severe or critical COVID-19 cases 7 days after the full vaccination; 2) The number of COVID-19 cases of any severity after the first dose of vaccination; 3) The number of COVID-19 cases of any severity in different age groups (18-59 years old, 60 years old and above) 7 days after the full vaccination.	
13.1 Study hypothesis			
5	**Main research hypothesis** At least 14 days after the full course of immunization, the lower limit of the 95% confidence interval (CI) of the protection rate to prevent COVID-19 of any severity is greater than 30% compared with placebo.	**Main research hypothesis** At least 7 days after the full course of immunization, the lower limit of the 95% confidence interval (CI) of the protection rate to prevent COVID-19 of any severity is greater than 30% compared with placebo.	Same revision description as above
13.3 Analysis Set			
6	Modified Full Analysis Set for Efficacy (E-mFAS): a subset of E-FAS, including all subjects who have completed the full vaccination and had at least one case monitoring follow-up 14 days after the full vaccination. Per-Protocol Set for Efficacy (E-PPS): including all subjects who have entered the randomization in accordance with the enrollment/exclusion criteria, completed the full vaccination and had at least one case monitoring follow-up 14 days after the full vaccination, without seriously violating the protocol. The morbidity in E-mFAS and E-PPS is counted starting from 14 days after the full course of vaccination, which is mainly used to	Modified Full Analysis Set for Efficacy (E-mFAS): a subset of E-FAS, including all subjects who have completed the full vaccination and had at least one case monitoring follow-up 7 days after the full vaccination. Per-Protocol Set for Efficacy (E-PPS): including all subjects who have entered the randomization in accordance with the enrollment/exclusion criteria, completed the full vaccination and had at least one case monitoring follow-up 7 days after the full vaccination, without seriously violating the protocol. The morbidity in E-mFAS and E-PPS is counted starting from 7 days after the full course of vaccination, which is mainly used to	Same revision description as above

	evaluate the main protective efficacy of the vaccine; the morbidity in E-FAS and E-mFAS1 is counted starting from after the first dose of vaccination.	evaluate the primary protective efficacy of the vaccine; the morbidity in E-FAS and E-mFAS1 is counted starting from after the first dose of vaccination.	
13.4.3 Evaluation of protective efficacy			
7	**13.4.3.1 Evaluation of the primary protective efficacy** Calculate the person-year incidence of COVID-19 of any severity and its 95% confidence interval starting from 14 days after the full vaccination, for the vaccine group and the placebo group respectively. Use Poisson Regression Model to statistically compare the differences between groups, and estimate the vaccine protection rate based on the person-year incidence and its 95% confidence interval according to the model. The Poisson Regression Model takes the number of cases as the dependent variable, the center, age group (18 to 59 years old, 60 years old and above) and grouping as the fixed effects, and the person-years of exposed subjects as the offset, using the log link function. If the person-year incidence of the vaccine group or the placebo group is close to 0, the exact probability test is used to calculate the 95% confidence interval of the protection rate based on the person-year incidence. Among them, the person-year incidence rate = (number of cases/person-years of exposed subjects) × 100%. In the calculation of person-years of exposure, the starting time is 14 days after the full course of vaccination, the termination time of COVID-19 case of any severity is the time when the case is first determined, and	**13.4.3.1 Evaluation of the primary protective efficacy** Calculate the person-year incidence of COVID-19 of any severity and its 95% confidence interval starting from 7 days after the full vaccination, for the vaccine group and the placebo group respectively. Use Poisson Regression Model to statistically compare the differences between groups, and estimate the vaccine protection rate based on the person-year incidence and its 95% confidence interval according to the model. The Poisson Regression Model takes the number of cases as the dependent variable, the center, age group (18 to 59 years old, 60 years old and above) and grouping as the fixed effects, and the person-years of exposed subjects as the offset, using the log link function. If the person-year incidence of the vaccine group or the placebo group is close to 0, the exact probability test is used to calculate the 95% confidence interval of the protection rate based on the person-year incidence. Among them, the person-year incidence rate = (number of cases/person-years of exposed subjects) × 100%. In the calculation of person-years of exposure, the starting time is 7 days after the full course of vaccination, the termination time of COVID-19 case of any severity is the time when the case is first determined, and	Same revision description as above

	the termination time for other subjects is the time point of their last protective efficacy follow-up. Vaccine protection rate = 1-(person-year incidence in the vaccine group/person-year incidence in the placebo group). Based on E-mFAS and PPS, evaluate the protective efficacy against COVID-19 of any severity after 14 days of full vaccination.	the termination time for other subjects is the time point of their last protective efficacy follow-up. Vaccine protection rate = 1-(person-year incidence in the vaccine group/person-year incidence in the placebo group). Based on E-mFAS and PPS, evaluate the protective efficacy against COVID-19 of any severity after 7 days of full vaccination.	
8	**13.4.3.2 Evaluation of the secondary protective efficacy** In addition, the statistical analysis method adopted to evaluate the protective efficacy against severe and critical COVID-19 after 14 days of full vaccination, and the protective efficacy against COVID-19 of any severity after at least one dose, is the same as the primary protective efficacy evaluation.	**13.4.3.2 Evaluation of the secondary protective efficacy** In addition, the statistical analysis method adopted to evaluate the protective efficacy against severe and critical COVID-19 after 7 days of full vaccination, and the protective efficacy against COVID-19 of any severity after at least one dose, is the same as the primary protective efficacy evaluation.	

A Randomized, Double-Blind, Placebo-Controlled Phase III Clinical Trial to Evaluate the Efficacy and Safety of Recombinant Novel Coronavirus Vaccine (CHO Cell) for the Prevention of COVID-19 in Subjects Aged 18 and Above

Sponsor:	Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.
Statistic Company:	Beijing Keytech Statistical Technology Co. Ltd.

Statistical Analysis Plan

Signed approval page

Statistic Company

Statistical analysis company: Beijing Keytech Statistical Technology Co. Ltd.

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Sponsor

Sponsor: Anhui Zhifei Longcom Biopharmaceutical Co., Ltd.

Person in charge of the Sponsor: Yang Shilong

Signature: Date:

Contents

1 Description of Abbreviations and Statistics Used in This Plan

AE	Adverse Event
ASaT	All Subjects as Treated
COVID	Corona Virus Disease
DSMB	Data Safety Monitoring Board
EAC	Endpoint Assessmentor Adjudication Committee
E-FAS	Full Analysis Set for Efficacy
E-mFAS	modified Full Analysis Set for Efficacy
E-PPS	Per-Protocol Set for Efficacy
GMT	Geometric Mean Titer
I-FAS	Full Analysis Set for Immunogenicity
I-PPS	Per-Protocol Set for Immunogenicity
IPS	Immunonegity Persistence Set
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ITT	Intend to Treat
IWRS	Interactive Web Response System
LOCF	Last Observation Carried Forward
Max	Maximum value
Mean	Mean value
Median	Median value
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum value
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
PT	Preferred Term
SAP	Statistical Analysis Plan
SARS-CoV	Severe Acute Respiratory Syndrome coronavirus
SD	Standard Deviation
SOC	System Organ Class
SS	Safety Set
TEAE	Treatment-Emergent Adverse Event

2 Introduction

This document was a Statistical Analysis Plan (SAP) for "the Randomized, Double-blind, Placebo-controlled Phase III Clinical Trial of the Efficacy and Safety of Recombinant Novel

Coronavirus Vaccine (CHO Cell) for the Prevention of COVID-19 in People Aged 18 Years and Older (V1.3)" , which mainly described the specific statistical analysis methods used to analyze and report on the baseline characteristics of subjects, evaluation of efficacy, immunogenicity and safety. The results of the statistical analyses from this study might be used for potential registration submissions for this product.

This statistical analysis plan shall be finalized and approved prior to database lock, and the corresponding statistical analysis programming shall be refined as the study data accumulates until database lock.

A sample statistical analysis form for this SAP would be provided separately as an attachment.

2.1 Analysis objective

The primary objective of this SAP is to evaluate the efficacy and safety of Recombinant Novel Coronavirus Vaccine (CHO Cell) in preventing COVID-19 of any severity in people aged 18 and above. The results of the corresponding statistical analyses will be presented in the final statistical report and clinical summary report, and will also be used for registration filings of this product, article publication, and other clinical needs.

Post-hoc exploratory analys can further explore the study data, but was not addressed in this SAP due to unpredictability. The corresponding statistical analysis methods for post-hoc exploratory analysis, if it occurs, will be detailed in the final statistical analysis report and clinical summary report.

Additional analyses for other purposes, such as publication of articles, regulatory or sponsor requests, were also not addressed in this SAP because they could not be anticipated. The corresponding statistical analysis method for the additional analysis, if they occurred, might not be detailed in the final clinical summary report, but would be detailed in the document presenting the additional results.

2.2 Modifications in the statistical analysis comparison protocol

The following additions had been made to the statistical analysis comparison protocol planned in this SAP:

(1) The primary efficacy analysis was performed using an exact Poisson regression model accounting for stratification. If the number of cases at either stratification level in the vaccine or placebo groups was 0, the exact method in StatXact software considering stratification factors was used to calculate the 95% confidence interval for the efficacy rate.

(2) Efficacy rate analysis based on person-year incidence in Poisson regression models (without accounting for stratification factors), efficacy rate analysis based on incidence rate, and efficacy rate analysis based on the Cox regression model were added to conduct sensitivity analysis of primary and secondary protection efficacy endpoints.

(3) Secondary efficacy endpoints were added: analysis of efficacy 14 days after full vaccination and 14 days after vaccination with at least one dose of vaccine, additional analysis of the efficacy of the vaccine was performed. For specific analysis indicators, please refer to Section 7.4.2 of the Statistical Analysis Plan.

(4) This clinical study focused on collecting cases through active and passive monitoring of patients. However, the case monitoring approach adopted varied slightly depending on the actual situation in different countries. Therefore, the actual case follow-up and recording had certain limitations, and the analysis set of efficacy was now modified, as detailed in section 6.1 of the statistical analysis plan. A sensitivity analysis of the primary efficacy was also performed using an exact Poisson regression model considering stratification factors based on the efficacy rate at the date of last case follow-up.

3 Research Objective

3.1 Primary objective

To evaluate the efficacy and safety of Recombinant Novel Coronavirus Vaccine (CHO Cell) in preventing COVID-19 of any severity in people aged 18 and above.

3.2 Secondary objective

- ✓ To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell)

against the severity of severe and above COVID-19 in a population aged 18 years and above.

- ✓ To evaluate the immunogenicity and immune persistence of the Recombinant Novel Coronavirus Vaccine (CHO Cell) in a population aged 18 years and above.
- ✓ To evaluate the efficacy of emergency vaccination of Recombinant Novel Coronavirus Vaccine (CHO Cell) in prevention of any severity of COVID-19 in the population aged 18 years and above.
- ✓ To evaluate the efficacy of the Recombinant Novel Coronavirus Vaccine (CHO Cell) against any severity of COVID-19 in populations of different age group (18-59 years, 60 years and above).

3.3 Exploratory objective

To explore the immunological alternative indicators of Recombinant Novel Coronavirus Vaccine (CHO Cell) in preventing COVID-19 in people aged 18 and above.

4 Trial Design

4.1 Overall design

It is a randomized, double-blind, placebo-controlled multi-regional clinical trial and a total of 29,000 subjects aged 18 or older are plan to be recruited, including 750 subjects aged 18-59 and 250 subjects aged 60 or older in China, and 21,000 subjects aged 18-59 and 7,000 subjects aged 60 or older outside China. For the subjects recruited in China, the safety and immunogenicity evaluation would be carried out, and for the subjects outside China, the efficacy, immunogenicity and safety evaluation would be conducted, and based on the immunogenicity evaluation, 750 subjects aged between 18 and 59 years and 250 subjects aged 60 years or older recruited outside China as well as all subjects in China will be selected as the immunogenicity subgroup for the purpose of immunogenicity bridging study. In the efficacy study cohort, the immunogenicity subgroup will be set, with a total of 1,000 subjects, and 500 subjects would be inoculated with study vaccines and another 500 subjects with placebos (the subjects from the immunogenicity subgroup would participate in the efficacy evaluation at the same time). Meanwhile, the immunogenicity study cohort should be set in China, with a total

of 1,000 subjects, and 500 subjects will be inoculated with study vaccines and another 500 subjects with placebos. Blood will be separately collected before vaccination, 14 days after full vaccination and 6 months after full vaccination to test the SARS-CoV-2 neutralizing antibody and RBD protein binding antibody and evaluate immunogenicity and immune persistence.

Table 1 Distribution of subjects

Region	Age group	Immunogenicity evaluation	Safety evaluation	Efficacy evaluation	Immunization procedure
China	18-59 years of age (750 cases)	All subjects	All subjects	None	0m, 1m & 2m
	60 years old and above (250 cases)				
Excluding China	18-59 years of age (21,000 cases)	750 cases	All subjects	All subjects	0m, 1m & 2m
	60 years old and above (7000 cases)	250 cases			

4.2 Criteria on study suspension or termination

In case that new data on the trial vaccine in this study or any other study was available, and the regulatory authorities, sponsor, investigator, and/or institutional review board/independent ethics committee believes that the trial should be suspended/terminated.

- **Trial suspension criteria:**

In case of the following conditions, the study needs to be suspended, and the institutional review board/independent ethics committee, relevant regulatory authorities will be reported immediately, data safety monitoring board (DSMB) expert meeting shall be held urgently for safety demonstration, analysis and determination on whether to continue this study.

Table 2 Trial suspension criteria

Adverse event leading to trial suspension	Number of cases/%
Vaccine-related deaths or serious life-threatening adverse reactions occurred	≥1 case

during the study period	
Adverse reactions ≥ grade 3 and lasting for 48 hours after any dose of vaccination	>15% of vaccinated population

- **Trial termination criteria**

The trial should be terminated and reported immediately to the Institutional Review Board/Independent Ethics Committee, relevant regulatory authorities in the following circumstances

Table 3 Trial termination criteria

Events leading to trial termination	**Number of cases/%**
Adverse reactions ≥ grade 3 and lasting for 48 hours after any dose of vaccination	>30% of vaccinated population

If the study was suspended or terminated prematurely, the sponsor would inform investigators, the ethics committee and drug regulatory authorities of the reason for the suspension or termination immediately in accordance with the requirements in corresponding registration regulations.

Regardless of the reason why the study was terminated in advance, the researcher should immediately inform the subjects and ensure that the subjects were properly followed up.

4.3 ADE/VED (antibody dependence enhancement/ vaccine enhanced disease) risk monitoring

After vaccination (at least 1 dose of the study vaccine), the subjects shall visit the hospital for hospitalization or isolation according to the local epidemic prevention and control requirements in case of confirmed COVID-19. For severe/critical cases/deaths a thematic investigation is required and DSMB will analyze the presence of ADE/VED based on the results of the thematic investigation.

4.4 Monitoring of COVID-19 cases

4.4.1 Diagnostic criteria and definition

(1) COVID-19 suspected cases: The one who experiences any of the following conditions: fever, cough, expectoration, shortness of breath, chill, fatigue, myalgia, pharyngalgia, nasal

congestion, headache, diarrhea, anorexia/nausea/vomiting, and loss of smell/ taste.

(2) COVID-19 cases: Samples should be taken twice for suspected cases (samples should be collected in duplicate each time, one for testing and one for backup and preservation), at a sampling interval of 24-48 h. The result of at least one SARS-COV-2 nucleic acid test by real-time RT-PCR should be positive and the patient should have any of the following conditions: fever, cough, expectoration, shortness of breath, chill, fatigue, myalgia, pharyngalgia, nasal congestion, headache, diarrhea, anorexia/nausea/vomiting, and loss of smell/taste.

(3) Mild COVID-19 cases: symptomatic patients with confirmed COVID-19 and no evidence of viral pneumonia or hypoxia. Symptoms including: fever, cough, fatigue, anorexia, shortness of breath, myalgia, sore throat, nasal congestion, headache, diarrhea, nausea, vomiting, loss of smell (anosmia), loss of taste (ageustia).

(4) Common COVID-19 cases: patients diagnosed with COVID-19, with clinical signs of pneumonia (fever, cough, dyspnea, shortness of breath, but no signs of severe pneumonia, including SpO2≥ 90% in indoor air conditions.

(5) Severe or critical COVID-19 cases: including a) and b)

a) Severe COVID-19 cases: patients diagnosed with COVID-19, with one of the following symptoms: ① shortness of breath, RR≥30 bpm; ② At resting state, SpO2＜90% in indoor air conditions; ③ Partial arterial oxygen pressure (PaO2)/fraction of inspired oxygen (FiO2)≤300mmHg (1mmHg=0.133kPa); in high-altitude areas (at an altitude of over 1,000 meters above the sea level), PaO2/ FiO2 shall be corrected by the following formula: PaO2/ FiO2 x[760/Atmospheric pressure (mmHg)]; and ④ The clinical symptoms were progressively aggravated, and the lung imaging showed that the lesion had significantly progressed over 50% within 24-48 hours.

b) Critical COVID-19 cases: confirmed COVID-19, respiratory failure (high-flux oxygen therapy, non-invasive ventilation, mechanical ventilation ECMO), shock, ICU admission, sepsis, combined acute pulmonary embolism, combined acute coronary syndrome, combined acute stroke, combined delirium, combined other organ failure, and death.

4.4.2 Case monitoring, diagnosis and treatment

4.4.2.1 Diagnostic procedure for suspected cases

COVID-19 case monitoring was symptom-driven passive monitoring. Prior to the start of the trial, the investigator should establish various feasible communication channels including internet, telephone, SMS and in-person visits to ensure that subjects could contact the investigator at any time, be familiar with the COVID-19 case monitoring and diagnosis process, and were strictly personal protective. Unified standard operating procedures (SOPs) were developed for all SARS-CoV-2 nucleic acid testing and training was provided to laboratory staff to ensure the authenticity and reliability of results and to reduce discrepancies.

If a subject suffer from symptoms as described in the definition of COVID-19 suspected case after the first dose of vaccination, the following procedures would be initiated:

1. The subject shall immediately contact the investigator (face-to-face interview, telephone, SMS, Internet).
2. The investigator shall confirm whether the definition of suspected case is met and shall complete a case monitoring form based on the symptom information provided by the subject.

(1) If the symptom meets the requirements, under the direction of the investigator, the subject shall arrive at the institution where the study is conducted or at a designated medical facility as soon as possible for the following tests.

Two nasopharyngeal swabs shall be collected by the site investigator (two swabs were collected for each sampling). One shall be subjected to real-time RT-PCR and the other shall be backed up and stored. Besides, respiratory rate (RR), oxygen saturation (SpO2), and oxygenation index (PaO2/FiO2) shall be tested. The investigators shall collect RT-PCR test report forms, diagnostic source documents, test report of S gene mutation loci and relevant test reports from all subjects with positive PCR tests:

- ✧ If the result of RT-PCR test is positive, the investigator shall conduct the COVID-19 case judgement based on the relevant symptoms and test results of the subjects. If the case is determined to be a COVID-19 case, it shall be taken care of according to the local epidemic diagnosis and treatment measures, and samples shall be taken again for

testing within 24-48 hours. The primary efficacy endpoint cases shall also be tested for SARS-CoV-2 S gene mutation loci by ARMS-PCR, and the main mutation loci detected were N501Y, A570D, HV69-70del, K417N, K417T, E484K.

- ✧ If the RT-PCR test is negative, the test shall be performed by resampling at an interval of 24-48 hours and respiratory rate (RR), oxygen saturation (SpO2), and oxygenation index (PaO2/FiO2) shall be tested. ① If the test result was positive, the investigator should judge whether it is COVID-19 case based on the related symptoms and test results of the subjects. Simultaneously, the primary efficacy endpoint cases shall also be tested for SARS-CoV-2 S gene mutation loci. ② If the test result is negative, the subject would be followed up by the investigator, and if the original symptoms persist or worsen within 2 days or if new symptoms of the suspected case of interest appear, case monitoring shall be performed and the sample shall be tested again according to the contents specified in (1); and follow-up shall be terminated if the original symptoms of the subjects disappear during the follow-up and there are no new symptoms related to suspected cases.

For suspected cases, if they are severe patients, the diagnosis shall not be ruled out based on a single negative upper respiratory tract sample, and additional upper respiratory tract and lower respiratory tract samples (sputum, airway extract and alveolar lavage fluid) should be collected. Among them, lower respiratory tract samples have higher positive detection rate and longer duration than upper respiratory tract samples. When these samples shall be available (for example, in patients on mechanical ventilation), investigators might choose to collect only lower respiratory tract samples.

(2) If not, the investigator would give some advice, such as routine medical treatment.

4.4.2.2 COVID-19 severity determination

Subjects diagnosed with COVID-19 should visit the local medical facility or designated medical facility for treatment according to the local treatment protocol as far as possible if hospitalization is required. The investigator should collect relevant clinical data during the hospitalization of the subjects and fill in the case monitoring form; if not hospitalized, the

subject should be taken care of according to local epidemic treatment measures, and home care should be done with good personal and family epidemic protection measures. The investigator could follow up the subject daily through face-to-face visits or by telephone, SMS, or internet, collect information on the course of the disease until the symptoms were reduced and fill in the case monitoring form, and if the subject develop respiratory distress during the follow-up process, the investigator need to remind the subject to go to the hospital for treatment immediately.

The investigators could determine the severity based on information about the course of disease or the presence of information that met the criteria for critical illness. (Note: If the subjects were not in critical condition, they should be followed up until the outcome, and the severity could be determined according to the most serious node in the course of disease based on the definition of COVID-19 severity. If the subjects are in critical condition, it meant the determination node have been reached, and the severity could be determined without waiting for its outcome).

Time of disease onset: time of first sign or symptom at the time of first laboratory confirmation of COVID-19. For example, if a subject reported to the investigator the onset of cough and fever on August 1, and the laboratory confirm the diagnosis of COVID-19 on August 3, the onset of disease in that case should be recorded as August 1.

Time of the disease end: the time when the disease is cured (with reference to the criteria for cure in each country); in the event of death, the time of death shall be recorded as the end time; if the disease is not cured at the end of the trial, the end time shall be left blank, and the outcome shall be recorded as "ongoing".

4.4.2.3 COVID-19 case review

- **COVID-19 case confirmation review**

The investigators shall collect clinical data from all subjects whose PCR tests are positive and submit the data to the Endpoint Assessmentor Adjudication Committee (EAC), which would review the COVID-19 case in accordance with the procedure set forth in the EAC's statutes. At the same time, under the premise of conforming to the policies of target countries,

an independent third party could be invited to review the positive samples in response to review requirements. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.

- **Confirmation review of the severity of COVID-19 cases**

The investigators shall collect all clinical data from the follow-up visits of confirmed COVID-19 cases and submit the data to the EAC, which would review the severity of the COVID-19 case in accordance with the procedures set forth in the EAC's protocols. If the results of the investigators and EAC are inconsistent, the EAC shall prevail.

4.4.3 Time of case monitoring

All vaccinated subjects shall be monitored for COVID-19 during the trial. The data on COVID-19 monitored from the 1st dose of vaccination to one year after the full course of vaccination would be statistically analyzed, and used for the registration application of the vaccine.

4.4.4 Collection of biological samples

1) The biological samples of nasopharyngeal swabs shall be collected from subjects reported as suspected COVID-19 cases at each site.

2) Biological samples collected from each center shall be divided into two copies and one is sent to the SARS-CoV-2 nucleic acid testing laboratory for real-time fluorescence quantitative RT-PCR and/or SARS-CoV-2 S gene mutation loci testing; and the other is kept in a qualified laboratory for backup.

Table 4 Study process of subjects

Visit	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	P11
Visit days (d)	**D-7~0**	**D0**	**V2+8**	**V2+30**	**V4+8**	**V4+30**	**V6+8**	**V6+14**	**V6+30**	**V6+180**	**V6+360**
Window period (d)	/	/	/	+7	/	+7		+7	/	+30	/
Informed consent	X										
Demographic data	X										
Medical history, allergy history	X										
History of recent medication (vaccination)	X										
Vital signs (axillary/oral temperature, blood pressure, pulse)		X									
Physical examination	X										
Axillary/oral temperature				X		X					
SARS-CoV-2 RT-PCR[5]	X										
SARS-CoV-2 antigen testing [6]	X										
SARS-CoV-2 IgG and IgM	X										
Urine pregnancy test		X									
Verification of inclusion and exclusion criteria	X	X									
Randomisation		X									
Vaccination		X		X		X					
Observation for 30 minutes after vaccination		X		X		X					

Distribute thermometers, measuring tapes, diary cards and organize training [3]		X		X		X					
Recycle diary cards and issue contact cards			X		X		X				
Recycle contact cards				X		X			X		
Concomitant medications [4]	X	X	X	X	X	X	X	X	X	X	X
Immunology blood collection[1]		X						X		X	
Reported serious adverse events		X	X	X	X	X	X	X	X	X	X
Reported pregnancy events		X	X	X	X	X	X	X	X	X	X
Monitoring of COVID-19 cases [2]		X	X	X	X	X	X	X	X	X	X

1 Immunological blood collection was performed only on 750 subjects aged 18-59 years and 250 subjects aged 60 years and older outside of China as well as all subjects in China.

2 If suspected cases of COVID-19 were found after the inoculation for the first dosing, nasopharyngeal swab was collected for RT-PCR test.

3 At Visit 2, the thermometer, straight ruler and diary card were granted; at Visit 4 and Visit 6 the diary cards were granted.

4 Only the concomitant medications for treating SAE and pregnant complications were collected from 30 days to 12 months after full vaccination.

5 SARS-CoV-2 real-time quantitative fluorescence RT-PCR assay was only available for subjects outside of Indonesia

6 SARS-CoV-2 antigen testing was only available for subjects in Indonesia.

4.5 Randomization and blinding

4.5.1 Randomisation

Stratified block randomization is adopted in this trial, subjects are stratified by center and age (18~59 years old, 60 years old and above), randomized allocation of subjects and study vaccines are completed using Interactive Web Response System (IWRS). SAS 9.4 or above version software is used by randomization statisticians to generate subject randomization table and vaccine randomization table, which are imported into IWRS system by system engineer. Investigators at each center participating in this trial shall log into the IWRS system to obtain the randomization numbers of the subjects after screening them; investigators log into the IWRS system to obtain the vaccine number prior to vaccination and vaccinate the subjects according to the vaccine number; in case of vaccine breakage, or the like, the investigator could obtain a new vaccine number from the IWRS system and take a new vaccine for vaccination.

4.5.2 Blinding

Prior to the start of the study, the staff from the sponsor who are not involved in this clinical study would blind the study vaccine uniformly together with non-blind randomization statisticians, i.e., pasted the printed label at the designated position of each vaccine according to the content of vaccine blindness. The randomization statistician supervise the blinding of vaccines, and guide the blinding operators to label according to the blind code. Upon completion of blinding, the content of blindness should be sealed by non-blind randomization statisticians. The entire blinding process shall be recorded and written down in a document form, the blinding record, which would be kept as one of the important documents of this clinical study. Blinders shall not participate in other related work of this clinical trial, and shall not disclose the blind code to any person participating in the clinical trial.

4.6 Sample size

4.6.1 Sample size calculation based on efficacy study

A large-scale confirmatory clinical study shall be required to evaluate the efficacy of the vaccine on COVID-19 in people aged 18 and above. The power is calculated assuming that the

incidence of COVID-19 with any severity is 1% during the trial. The trial is planned to perform separate interim analysis when 1/3 and 2/3 of COVID-19 cases of any severity are observed, using the O'Brien-Fleming alpha-spending function to keep the total type I error of the trial within 5% (bilaterally). To test 60% vaccine efficacy (lower 95% CI >30%), a total of 156 COVID-19 cases of any severity and 22,144 subjects (11,072 subjects per group) were required to achieve 90% certainty. The calculation of the number of events is based on the exact condition method under the assumption of Chan and Bohidar's large sample Poisson distribution. Therefore, 14,000 subjects shall be recruited in each group under the comprehensive consideration of dropout, protocol deviation, and non-compliance.

4.6.2 Sample size calculation based on immunogenicity bridging study

Set a non-inferiority margin of 0.67, one-sided α= 0.025 as the test level, and a power of 90%, and assume that the GMT of anti-SARS-COV-2 neutralizing antibody in people in China and outside China is the same, the standard deviation of the antibody titer after logarithmic conversion is 0.55, and the distribution ratio of the two groups of samples is 1:1. Using PASS 15, the minimum sample size of each study vaccine group in China and outside China is 207. Considering factors such as shedding and age distribution, 1000 subjects are planned to be enrolled in each study vaccine group in China and outside China (750 subjects aged 18-59 and 250 subjects aged 60 and above). Therefore, a total of 2,000 cases are enrolled, including 1,000 cases in China and 1,000 cases outside China (1,000 cases abroad are immunogenicity subgroups of the protective efficacy study cohort and participate in the efficacy evaluation), 1,000 cases in the study vaccine group and 1,000 cases in the placebo group.

In conclusion, the total sample size of phase III shall be 29,000 cases.

5 Evaluation Endpoints

5.1 Efficacy evaluation endpoints

5.1.1 Primary efficacy endpoints

(1) Efficacy against COVID-19 cases of any severity 7 days after full vaccination.

Vaccine efficacy rates are calculated based on person-year incidence and incidence rates, respectively. The specific calculation formulas are shown below:

- The vaccine efficacy rate based on the **person-year incidence** is calculated using the following formula:

$$\text{Efficacy rate (\%)} = \left(1 - \frac{\text{Person} - \text{year incidence of vaccine group}}{\text{Person} - \text{year incidence of placebo group}}\right) \times 100\%,$$

Where, person-year incidence = (number of patients/exposure person-years of subjects) ×100%.

- The **incidence**-based vaccine efficacy rate is calculated using the following formula:

$$\text{Protective efficacy rate (\%)} = \left(1 - \frac{\text{Incidence of vaccine group}}{\text{Incidence of placebo group}}\right) \times 100\%,$$

Where, incidence rate = (number of cases/number of subjects) × 100%.

The number of cases is defined as the number of COVID-19 cases determined by the Endpoint Events Committee (EAC).

A COVID-19 case 7 days after full vaccination is defined as: If a subject completed the 3rd (full) vaccination dose on March 1, 2020, and becomes infected with COVID-19 after March 9, 2020 (inclusive), he or she is counted as a COVID-19 case 7 days after full vaccination.

Person-years of exposure is defined as (end date of the monitoring period of the effective case - the date of 3rd dose - 7)/365.25; Where the **termination date** of the monitoring period of the effective case is calculated according to the following rules:

- ✓ For subjects diagnosed with COVID-19 before the data analysis deadline, the termination date is the date of first onset of symptom of COVID-19 (the date of first onset);
- ✓ for subjects tested positive by RT-PCR assays in the laboratory after vaccination but not diagnosed with COVID-19 before the data analysis deadline, the termination date is the earlier of the date of first RT-PCR positive result after vaccination or the data cut-off date or the date of receiving other COVID-19 vaccines;
- ✓ for subjects who are not withdrawn from the trial before the data analysis deadline, and not tested positive by RT-PCR assays in the laboratory or diagnosed with COVID-19, the termination date is the earlier of the data cut-off date or the date of receiving other COVID-19 vaccines;
- ✓ for subjects who have completed the trial before the data analysis deadline, and are

not tested positive by RT-PCR assays in the laboratory or diagnosed with COVID-19, the termination date is the earlier of the trial completion date or data cut-off date or the date of receiving other COVID-19 vaccines;

- ✓ for subjects who are withdrawn from the trial before the cut-off date for data analysis, and not tested positive by RT-PCR assays in the laboratory or diagnosed with COVID-19, the termination date is the earlier of the withdrawal from the trial or the data cut-off date or the date of receiving other COVID-19 vaccines.

5.1.2 Secondary efficacy endpoints

(1) Efficacy against of COVID-19 cases in different situations 7 days after full vaccination:

- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death;
- ✧ different COVID-19 variants included Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2/AY.4/AY .6/AY.12), Kappa (B.1.617.1/B.1.617.3) and Deltaplus/AY.1 (B.1.617.2.1).

(2) Efficacy against COVID-19 cases in different situations, 7 days after the inoculation of at least one dose of vaccine:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death.

(3) Efficacy against COVID-19 cases in different situations after the inoculation of at least one dose of vaccine:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death.

(4) Efficacy against COVID-19 cases in different situations 14 days after full vaccination:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death;

- ✧ different COVID-19 variants included Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2/AY.4/AY .6/AY.12), Kappa (B.1.617.1/B.1.617.3) and Deltaplus/AY.1 (B.1.617.2.1).

(5) Efficacy against COVID-19 cases in different situations 14 days after the inoculation of at least one dose of vaccine:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death.

5.2 Immunogenicity evaluation endpoints

5.2.1 Basic phase

- ➢ **The following immunogenicity endpoints are defined for SARS-CoV-2 neutralizing antibodies:**

(1) The positive rate of neutralizing antibody 14 days after full immunization: the titer of neutralizing antibody was ≥ 1:4 after immunization.

(2) Positive conversion rate of neutralizing antibody 14 days after full immunization of the population with negative pre-immunization test results: the positive conversion rate of neutralizing antibody 14 days after full immunization of the population with negative pre-immunization test results is defined as the titer of neutralizing antibody <1:4, and the titer of neutralizing antibody ≥1:4 14 days after full immunization in the population.

(3) The 4-fold growth rate of neutralizing antibody in the population with positive pre-immunization test results after 14 days of full immunization: The 4-fold increase in neutralizing antibody within 14 days after full immunization in the population with positive pre-immunization test results is defined as the titer of neutralizing antibody ≥1:4 before immunization, and the titer of neutralizing antibody 14 days after the full immunization for the population was 4-fold or more than before immunization.

(4) The rate of positive neutralizing antibody (4-fold increase) 14 days after full immunization: where positive neutralizing antibody (4-fold increase) 14 days after full immunization is defined as those with neutralizing antibody titer <1:4 before immunization and those with neutralizing antibody titer ≥1:4 14 days after full immunization; Or if the titer of

neutralizing antibody before immunization was ≥1:4, and increased more than 4 times compared with that before immunization after 14 days of full immunization.

(5) Neutralizing antibody A1 GMT 14 days after full immunization and its increase compared with that before immunization.

(6) Neutralizing antibody GMT and its increase over the pre-immunization period 14 days after the full immunization for the population with negative pre-immunization test results.

(7) Neutralizing antibody GMT and its increase over the pre-immunization period 14 days after the full immunization for the population with positive pre-immunization test results

- **The following immunogenicity endpoints are defined for RBD protein binding antibody IgG antibody:**

(1) The positive rate of binding antibody 14 days after full immunization: the title of binding antibody is ≥ 1:11 after immunization.

(2) Positive conversion rate of binding antibody 14 days after full immunization of the population with negative pre-immunization test results: the positive conversion rate of the binding antibody 14 days after full immunization of the population with negative pre-immunization test results is defined as the titer of binding antibody <1:11, and the titer of binding antibody ≥1:11 14 days after full immunization in the population.

(3) The 4-fold growth rate of binding antibody in the population with positive pre-immunization test results after 14 days of full immunization: The 4-fold increase in binding antibody within 14 days after full immunization in the population with positive pre-immunization test results is defined as the titer of binding antibody ≥1:11 before immunization, and the titer of binding antibody 14 days after the full immunization for the population was 4-fold or more than before immunization.

(4) The positive conversion rate of binding antibody (4-fold increase) 14 days after full immunization: where positive conversion rate of binding antibody (4-fold increase) 14 days after full immunization was defined as those with binding antibody titer <1:11 before immunization and those with binding antibody titer ≥1:11 14 days after full immunization; or if the titer of binding antibody before immunization is ≥1:11, and increased more than 4 times compared with that before immunization after 14 days of full immunization.

(5) Binding antibody GMT 14 days after full immunization and its increase compared with that before immunization.

(6) Binding antibody GMT and its increase over the pre-immunization period 14 days after the full immunization for the population with negative pre-immunization test results.

(7) Binding antibody GMT and its increase over the pre-immunization period 14 days after the full immunization for the population with positive pre-immunization test results.

5.2.2 Phase of immune persistence

The following immunogenicity endpoints were defined for SARS-CoV-2 neutralizing antibody, RBD protein binding antibody and IgG antibody, respectively:

(1) The positive rate of antibody 6 months after full vaccination.

(2) Antibody GMT 6 months after full vaccination.

5.3 Safety endpoints

(1) Adverse events including

- **Solicited adverse events**: This test collected adverse events within 7 days after each dose of vaccination, including adverse events at the injection site (local) and uninjection site (systemic) adverse events. Among them, adverse events at the injection site (local) : including pain, swelling, induration, redness, rash, and itching. Adverse events at uninjection site (systemic): including headache, fatigue/tiredness, nausea, vomiting, diarrhea, muscle pain (uninjection site), cough, acute allergic reaction, mental disorder (specific symptoms), and fever.
- **Unsolicited adverse events:** including other adverse events during the solicited period and all adverse events during the unsolicited period.

(2) Serious adverse events: including serious adverse events occurring within 12 months after the first dose of vaccination to the full vaccination.

6 Analysis Sets

6.1 Protective efficacy analysis sets

(1) Full Analysis Set for Efficacy (E-FAS): including all subjects who were randomized to receive at least one dose of vaccination following the principles of intent-to-treat (ITT).

Subjects who were incorrectly vaccinated were analyzed for efficacy according to their allocation after randomization based on the ITT principle. Full analysis set was used to evaluate the protective efficacy after the first dose in subjects who received at least one dose of vaccination. The endpoint cases were counted since the first dose of vaccination.

(2) E-mFAS (modified Full Analysis Set for Efficacy): It was a subset of E-FAS, including all subjects who completed full vaccination, while excluding:

- ✧ Subjects who failed to enter the case monitoring period due to dropout less than 7 days after the third dose of vaccination;
- ✧ Subjects who received other Covid-19 vaccines less than 7 days after the third dose of vaccination;
- ✧ Subjects who were less than 7 days after the third dose of vaccination;
- ✧ Subjects who were RT-PCR tested as SARS-CoV-2 positive between the first dose and 7 days after the third dose of vaccination;
- ✧ Subjects who were diagnosed as Covid-19 cases between the first dose and 7 days after the third dose of vaccination;
- ✧ Subjects who did not complete three doses of vaccination;
- ✧ Subjects with positive RT-PCR or positive antigen test or positive antibody test for SARS-CoV-2 at baseline during the screening period.

(3) E-PPS (Per-Protocol Set for Efficacy): including all subjects who did not violate the inclusion/exclusion criteria, and received the full vaccination (3 doses) after randomization, and did not obviously violate the protocol, but excluding:

- ✧ Subjects who failed to enter the case monitoring period due to dropout less than 7 days after the third dose of vaccination;
- ✧ Subjects who received other Covid-19 vaccines less than 7 days after the third dose of vaccination;
- ✧ Subjects who were less than 7 days after the third dose of vaccination;
- ✧ Subjects who were RT-PCR tested as SARS-CoV-2 positive between the first dose and 7 days after the third dose of vaccination;
- ✧ Subjects who were diagnosed as Covid-19 cases between the first dose and 7 days after the third dose of vaccination;
- ✧ Subjects who were vaccinated incorrectly or did not complete three doses of vaccination;

- ✧ Vaccination who received the doses outside of window;
- ✧ Subjects with positive RT-PCR or positive antigen test or positive antibody test for SARS-CoV-2 during at baseline during the screening period.

Cases in E-mFAS and E-PPS were calculated from 7 days after full vaccination for the evaluation of the primary efficacy of the vaccine. E-mFAS was the main data set for the evaluation of vaccine efficacy.

6.2 Immunogenicity analysis sets

(1) Full Analysis Set for Immunogenicity: including all subjects who followed the ITT principles, completed randomization and finished at least one dose of vaccination, and had valid immunogenicity results before immunization. Subjects incorrectly vaccinated were subject to randomized grouping for immunogenicity evaluation according to the ITT principle.

(2) Per-Protocol Set for Immunogenicity (I-PPS): including all subjects who conformed to the inclusion and exclusion criteria, completed full vaccination of vaccine, and had valid immunogenicity results of both pre-immunization and 14 days after full vaccination.

(3) Immunonegity Persistence Set (IPS): including all subjects who completed blood sample collection for immune persistence evaluation 6 months after full vaccination, and had valid antibody data.

I-FAS and I-PPS were applied for immunogenicity analysis. IPS was used for the immune persistence analysis.

6.3 Safety analysis sets

(1) Safety Set (SS): All subjects who received at least one dose of the study vaccine. Any subjects with wrong vaccine numbers shall be subject to safety evaluation based on actual injected vaccine groups according to ASAT (All Subjects As Treated) principle.

The safety analysis sets would also define the first dose safety analysis set, the second dose safety analysis set and the third dose vaccine safety analysis set, among which the first dose safety analysis set included subjects who completed the first dose of vaccine for the analysis of safety after the first dose of vaccination; the second dose safety analysis set included subjects who completed the second dose of vaccine for the analysis of safety after the second dose of

vaccination; the third dose safety analysis set included subjects who completed the third dose of vaccine for the analysis of safety after the third dose of vaccination.

All analysis sets would be discussed by the principal investigator, the sponsor, the statistician and the data manager during a blind data review prior to database locking.

7 Statistical Analysis Method

7.1 General considerations

7.1.1 Hypothesis testing

- **Hypothesis test I:** The lower limit of the 95% confidence interval (CI) for efficacy rate against COVID-19 of any severity compared with placebo 7 days after full vaccination was greater than 30%. That was:

  Original hypothesis H_0: VE≤30%,

  Alternative hypothesis H_1: VE>30%,

  One-sided $\alpha = 0.025$ was taken as the test level, and the lower limit of CI for vaccine efficacy (VE) > 30%.

- **Hypothesis test II:** The anti-SARS-CoV-2 neutralizing antibody GMT 14 days after full immunization of the population with negative pre-immunization test results in the study vaccine group in China was not inferior to those outside China, that was:

$$\text{Original hypothesis} H_0: \frac{GMT_C}{GMT_O} \leq \Delta_2,$$

$$\text{Alternative hypothesis} H_1: \frac{GMT_C}{GMT_O} > \Delta_2,$$

where GMT_C, GMC_O denoted the post-immunization antibody GMT of the population with negative pre-immunization test results in China and outside China, respectively, and $\Delta_2 = 0.67$ was taken as the non-inferiority margin, one-sided $\alpha = 0.025$ was taken as the test level .

7.1.2 General analytical methods

- **Descriptive statistics**

Unless otherwise stated, the following descriptive statistics summary would be given according to the type of variables:

✓ Continuous variables were summarized using mean, standard deviation, median,

minimum and maximum values.

✓ Categorical or ordinal variables would be summarized using frequencies and percentages. The 95% CI for percentages were calculated using the Clopper-Pearson method.

The following descriptive statistics would also be used for titers and their growth multiples: geometric mean and 95% CI. Geometric mean titers were obtained by inverse logarithmic transformation of the mean of the logarithmic original titers. The 95% CI was obtained by constructing a 95% CI for the mean of the logarithmic raw titers using the grouped t-distribution method and then inverse logarithmic transformation of the upper and lower limits of the confidence interval to obtain the 95% CI for the titers at the original scale. The titer growth multiples were analyzed in a similar way.

➢ **Number of decimal places**

Unless otherwise specified, the following rules were applied to the decimal places in the analysis report:

✓ The minimum and maximum values had the same maximum number of decimal places as the original data;

✓ One more decimal place for the median, 25% and 75% quartiles, mean, geometric mean, standard deviation, and 95% CI than the maximum decimal place of the original data;

✓ percentages, ratios, and 95% CI for ratios were rounded to 2 decimal places;

✓ If the P-value was ≥ 0.0001, it was rounded to 4 decimal places; if the P value was <0.0001, it was reported as "<0.0001".

✓ Test statistics for all statistical tests were rounded to 3 decimal places.

✓ Derivative data were rounded to 2 decimal places.

7.1.3 Relavant definitions and derivation rules

➢ **Data analysis deadline**

The cut-off date for the first data analysis of this study was June 30, 2021.

➢ **Baseline**

Unless otherwise specified, "baseline" was defined as the last non-null test value before the first injection.

- **Year-month-day conversion**

Month = days/30.4375, year = days/365.25, rounded to one decimal place.

- **Adverse Event**

Duration of adverse event (days) = date of adverse event - date of injection for that adverse event dose.

Duration (days) = End date of adverse event - Start date of adverse event+1.

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

TEAE was defined as the adverse events occurring after the first vaccination (including the day of the first vaccination) or aggravated after the first vaccination, and was programmed according to the following rules: if the date of the adverse event occurred after the date of the first vaccination (including the same date), it was counted as TEAE; If the adverse event occurred before the first vaccination, it was counted as a non-TEAE; If the date of occurrence of the adverse event or the date of the first vaccination was missing so that it was not clear whether the adverse event occurred after the first vaccination, the adverse event shall be counted as TEAE.

- **Relevance of adverse events**

Relevant to the study vaccine meant that the adverse event was "possibly related", "probably related" or "definitely related" to the vaccination; not relevant to the study vaccine meant that the relationship between the adverse event and the vaccination was "definitely not relevant" or "probably not relevant".

7.1.4 Pre-existing diseases and co-morbidities

The following rules were used to determine pre-existing disease and comorbid disease, respectively:

- ✓ Pre-existing disease was defined as a disease that ended before the first vaccination.
- ✓ Comorbid disease was defined as disease that started before the first vaccination and ended after the first vaccination or was ongoing, or disease that started after the start

of the first vaccination.

7.1.5 Analysis window

For visits after the baseline, when analyzing by visit, statistical analysis would be performed according to the visit time points planned by the protocol, and time points not planned by the protocol would not be considered. The results of inspections not planned for the protocol would be listed in the checklist.

7.1.6 Analysis software

All statistical analysis shall be conducted by statistical software SAS 9.4 or versions above.

7.1.7 Form and list

- **Form**

Data were generally summarized separately by group (vaccine group and placebo group) and in immunogenicity bridging trials by China region and vaccine group outside of China region. Groups shall generally be presented as columns.

- **Lists**

Unless otherwise specified, all lists shall include group, subject number, and raw data in SDTM shall be presented preferentially. Lists are generally ordered by group, subject number, visit time, or other relevant time (e.g., time of AE occurrence).

7.2 Subject distribution

The screening population, screening failure population, randomized enrollment, completion of each dose of injection, withdrawal from the trial, in-progress and completion of the trial, and the number of subjects in each analysis set shall be summarized by country and site.

A list of subjects who withdrew from the trial and a list of subjects who do not enter each analysis set shall be presented separately.

List protocol violations/deviations.

7.3 Demographic data and baseline characteristics

Demographic data, baseline characteristics and other indicators are described statistically

as follows:

- ✓ Demographic data (including age, gender, ethnicity, height, weight, and body mass index);
- ✓ Clinical characteristics (including baseline SARS-CoV-2 status, IgG testing, IgM testing, PCR or antigen testing of SARS-CoV-2, case monitoring time after the first dose and the third dose of injection);
- ✓ Baseline vital signs (including body temperature, pulse, systolic blood pressure, and diastolic blood pressure);
- ✓ Baseline physical examination (including skin and mucous membrane, lymph node, head, neck, chest, abdomen, spine/ limbs).

According to the distribution characteristics of variables, the group T test shall be used to compare statistically the intergroup age, height, weight, body mass index, case monitoring time after the first dose and the third dose of injection, and quantitative indicators of vital signs and other variables. The Chi-square test /Fisher exact probability test shall be used to compare statistically the intergroup gender, race, IgG test, IgM test, PCR or antigen test of RT-SARS-CoV-2 and qualitative indicators of physical examination and other variables.

The WHODD Mar-2021 or the latest version shall be used to code the medical treatment history, vaccination history, concomitant medication and combined vaccination and to calculate the incidence, number of cases and utilization rate of each group, respectively. The Fisher exact probability test shall be used to compare statistically the intergroup differences.

The MedDRA24.0 or the latest version shall be used to code the past medical history and the combined diseases, and to calculate the incidence, number of cases and incidence rate of various past medical history and the combined diseases of each group, respectively. The Fisher exact probability test shall be used to compare statistically the intergroup differences.

The medication history, vaccination history, concomitant medication and vaccination, and past medical history and concomitant medical conditions shall be listed.

The analysis above is based on E-FAS and I-FAS, except that the concomitant medication and concomitant vaccination are based on SS, and the clinical characteristics is based on E-FAS,

E-mFAS, and E-PPS.

Demographic information and baseline characteristics shall be summarized by total, China and outside China respectively.

7.4 Efficacy evaluation

7.4.1 Analysis of primary efficacy

- **Efficacy in the COVID-19 cases of any severity at Day 7 after full vaccination**

The person-year incidence of COVID-19 of any severity diagnosed at Day 7 after full vaccination and its 95% CI shall be calculated in the vaccine group and placebo group. The exact Poisson regression model is used to compare statistically the intergroup differences, and the vaccine efficacy rate and its 95% CI based on person-year incidence shall be estimated based on the model. The log link function shall be used for the model with the number of patients as the dependent variable in the model, and the site, age group (18~59 years vs. 60 years old and above) and grouping as the fixed effect, and the exposure person-year number of subjects as the offset. If the number of cases at either stratification level in the vaccine or placebo groups was 0, the exact method in StatXact software considering stratification factors was used to calculate the 95% CI for the efficacy rate.

The Kaplan-Meier curves of COVID-19 cases of any severity diagnosed at Day 7 after full vaccination are plotted.

- **Sensitivity analysis 1 (efficacy rate analysis based on the last case follow-up date):**

In the calculation of the exposure person-year number , the data deadline or the last case monitoring and follow-up date before the date of early withdrawal from the trial (for subjects who withdraw from the trial earlier) shall be used as the termination time of the case monitoring period, and the person-year incidence and efficacy of each group shall be calculated by derivation. The statistical method of efficacy shall be the same as that in the primary analysis. The specific rules for the termination time of the case monitoring period are as follows:

- ✓ For subjects diagnosed with COVID-19 before the data analysis deadline, the termination time was the date of first onset of symptom of COVID-19 (the date of first onset);

- ✓ For subjects who were tested positive by RT-PCR assays in the laboratory but not diagnosed with COVID-19 after vaccination before data analysis deadline, the termination time was the date of the first RT-PCR positive result after vaccination or the data deadline date or the earlier date of injection of other COVID-19 vaccines;
- ✓ For subjects who did not withdraw from the trial before the data analysis deadline, and were not tested positive by RT-PCR assays in the laboratory or diagnosed with COVID-19, the termination time was the last follow-up date or the earlier date of injection of other COVID-19 vaccines;
- ✓ For subjects who had completed the trial before the data analysis deadline, and were not tested positive by RT-PCR assays in the laboratory or diagnosed with COVID-19, the termination time was the last follow-up date before the completion of the trial or the earlier date of injection of other COVID-19 vaccines;
- ✓ For subjects who did not withdraw from the trial before the data analysis deadline, and were not tested positive by RT-PCR assays in the laboratory and not diagnosed with COVID-19, the termination time was the last follow-up date before the withdrawal from the trial or the earlier date of injection of other COVID-19 vaccines.

- ➢ **Sensitivity analysis 2 (vaccine efficacy rate analysis without considering stratification factor):**

The Poisson regression model was used to calculate the vaccine efficacy rate and its 95% CI. The number of patients was taken as the dependent variable, the grouping as the fixed effect, the exposure person-year number of subjects as the offset in the model, and the log link function was used. The 95% CI for vaccine efficacy rate was calculated using the exact method in the StatXact software if the CI could not be estimated due to 0 case in any sub-group in the vaccine group or the placebo group.

- ➢ **Sensitivity analysis 3 (incidence rate-based vaccine efficacy rate analysis):**

The incidence rate of COVID-19 of any severity diagnosed at Day 7 after full vaccination and its clopper-Pearson 95% CI were calculated in the vaccine group and the

placebo group, respectively, and the inter-group differences were statistically tested using the χ^2test/Fisher's exact test; The efficacy rate of the study vaccine and its 95% CI were calculated according to the efficacy rate=1- (the incidence rate in the vaccine group/the incidence rate in the control group).

- **Sensitivity analysis 4 (Cox regression model-based vaccine efficacy rate analysis):**

The Cox proportional hazard model was used to fit with Cox proportional hazard model, with the exposure person-year number as the dependent variable, the center, age group (18-59 years vs. 60 years and above) and group as the fixed effects, and the vaccine efficacy rate (= 1-HR) and its 95% CI were calculated according to the model.

The algorithm for Cox regression model-based exposure person-year number was the same as that for the primary endpoint, i.e., the method for the efficacy of COVID-19 of any severity at Day 7 after the full vaccination.

The analysis above is based on E-mFAS and E-PPS. Wherein, E-mFAS is the primary analysis set for this protective efficacy evaluation.

7.4.2 Secondary Efficacy Assessments

(1) Efficacy against COVID-19 cases of different situations 7 days after full vaccination:

- ✧ severe and above (including severe and critical) COVID-19 cases, COVID-19 cases resulting in hospitalization and death;
- ✧ different COVID-19 strains included Alpha (British strain B.1.1.7), Beta (South African strain B.1.351), Gamma (Brazil strain P.1), Delta (Indian strain B.1.617.2/AY.4/AY .6/AY.12), Kappa (B.1.617.1/B.1.617.3) and Deltaplus/AY.1 (B.1.617.2.1).

The analysis above is based on E-mFAS and E-PPS.

(2) Efficacy against COVID-19 cases of different situations 7 days after receiving at least one dose of vaccine:

- ✧ COVID-19 cases of any severity; and
- ✧ severe and above (including severe and critical) COVID-19 cases, COVID-19 cases resulting in hospitalization and death.

- ✧ The analysis above is based on evaluable subjects in E-FAS, i.e, PCR positive or antigen test positive, or antibody to SARS-CoV-2 excluding baseline SARS-CoV-2 in E-FAS; The corresponding COVID-19 cases, withdrawal from the trial or PCR positive or vaccination with other COVID-19 vaccines occur within 7 days of the first dose vaccination; or subjects with their first dose less than 7 days prior to the analysis deadline date.
- ✧ Person-Years of Exposure = (the observation termination time of the effective case monitoring period-the first dose vaccination time -7)/365.25. The algorithm for the observation termination time of effective case monitoring period is the same as that for the primary endpoint, i.e., the efficacy of COVID-19 of any severity at Day 7 after the full-course vaccination.

(3) **Efficacy against COVID-19 cases of different situations after receiving at least one dose of vaccine:**

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death.
- ✧ The above analysis is based on evaluable subjects in E-FAS, i.e., positive PCR or antigen test or positive antibody to SARS-CoV-2 excluding baseline in E-FAS.
- ✧ The algorithm for the observation termination time of effective case monitoring period is the same as that for the primary endpoint, i.e., the efficacy of COVID-19 of any severity at Day 7 after the full-course vaccination.

(4) Efficacy against COVID-19 cases of different situations 14 days after full vaccination:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death;
- ✧ different COVID-19 strains included Alpha (British strain B.1.1.7), Beta (South African strain B.1.351), Gamma (Brazil strain P.1), Delta (Indian strain B.1.617.2/AY.4/AY .6/AY.12), Kappa (B.1.617.1/B.1.617.3) and Deltaplus/AY.1

(B.1.617.2.1).

- ✧ The above analysis is based on evaluable subjects in E-mFAS and E-PPS excluding subjects who have the corresponding COVID-19 within 14 days after full-course vaccination, withdraw from the trial, or have PCR-positive or other COVID-19 vaccination in E-mFAS and E-PPS; Or subjects with their third dose of vaccination less than 14 days prior to the analysis deadline date.
- ✧ Exposure person-year number = (the observation termination time of effective case monitoring period-the third dose of vaccination time -14)/365.25. The algorithm for the observation termination time of effective case monitoring period is the same as that for the primary endpoint, i.e., the efficacy of COVID-19 of any severity at Day 7 after the full-course vaccination.

(5) Efficacy against COVID-19 cases of different situations 14 days after receiving at least one dose of vaccine:

- ✧ COVID-19 cases of any severity;
- ✧ severe COVID-19 cases and above (including severe and critical COVID-19 cases), COVID-19 cases resulting in hospitalization and death;
- ✧ The analysis above is based on evaluable subjects in E-FAS, i.e., PCR positive or antigen test positive, or antibody to SARS-CoV-2 excluding baseline SARS-CoV-2 in E-FAS; The cases who develop corresponding COVID-19, withdraw from the trial or PCR positive or vaccinated with other COVID-19 vaccines within 14 days of the first dose of vaccination; Or subjects with their first dose less than 14 days prior to the analysis deadline date
- ✧ Exposure person-year number = (the observation termination time of effective case monitoring period-the first dose of vaccination time -14)/365.25. The algorithm for the observation termination time of effective case monitoring period is the same as that for the primary endpoint, i.e., the efficacy of COVID-19 of any severity at Day 7 after the full-course vaccination.

Based on the E-FAS analysis set, the vaccine efficacy rates and their 95% CIs are calculated for each of the different follow-up time periods after the first dose of vaccination.

The statistical analysis method for all secondary efficacy endpoint is same as that for primary efficacy evaluation endpoint.

7.5 Immunogenicity evaluation

7.5.1 Basic phase evaluation

The analysis below is based on I-FAS and I-PPS.

7.5.1.1 Immunogenicity evaluation in the immunogenicity bridging study

In the immunogenicity bridging study, the anti-SARS-CoV-2 neutralizing antibody GMT at Day 14 after full-course immunization of pre-immunization negative population in the test vaccine group is fitted with a covariance analysis model after logarithmic transformation for statistical comparison. The values of anti-SARS-CoV-2 neutralizing antibodies at Day 14 after full-course immunization after logarithmic transformation of pre-immunization negative population are taken as the dependent variable in the model, the logarithmic transformation values of anti-SARS-CoV-2 neutralizing antibodies before immunization as the covariates, and the participating countries (China vs. regions outside China) and different age groups (18–59 years old vs. 60 years old and above) as the fixed effects; According to the model, the least-squares mean and 95% confidence interval of the logarithmic transformation values and the inter-group difference of the anti-SARS-CoV-2 neutralizing antibody at Day 14 after full immunization of the pre-immunization negative population in China and regions outside China are calculated respectively; And after inverse logarithmic transformation, the least-squares mean and 95% confidence interval of the GMT ratio of the anti-SARS-CoV-2 neutralizing antibody GMT (in China/out of China) at Day 14 after full immunization of pre-immunization negative population in China and regions outside China are calculated. If the lower limit of the 95% confidence interval of the GMT ratio (in China/outside China) is greater than 0.67, the immunogenicity of the test vaccine group in China is considered to be non-inferior to the test vaccine group outside China.

The post-immunization antibody positive rate of anti-SARS-CoV-2 neutralizing antibody and RBD protein-binding antibody IgG antibody, the post-immunization antibody positive rate in the pre-immunization negative population, the post-immunization antibody positive

conversion rate in the pre-immunization positive population, the post-immunization antibody four-fold growth in the pre-immunization positive population and the post-immunization antibody positive conversion rate (four-fold growth) in all the populations of the vaccinated subjects in China and regions outside China in the immunogenicity bridging study are calculated respectively. The 95% confidence interval is calculated by using the Clopper-Pearson method. The difference in rates between the vaccine and placebo groups and its 95% CI are calculated using the Miettnen-Nurminen method and the difference between the vaccine and placebo groups is statistically tested using Chi-square test /Fisher's exact probability method.

The geometric mean and bilateral 95% confidence intervals are used to statistically describe the GMT and GMT increase folds of anti-SARS-CoV-2 neutralizing antibody, RBD protein-binding antibody IgG antibody at Day 14 after full immunization of all populations, pre-immunization negative populations and pre-immunization positive populations in the test vaccine group of the immunogenicity bridging study in China and regions outside China, respectively. The intergroup GMT ratio of antibodies at Day 14 after full immunization and its 95% confidence interval (China/regions outside China) are calculated, and the log-transformed group t-test shall be used to statistically test the differences between China and regions outside China.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody before immunization and at Day 14 after full-course immunization shall be plotted for China and outside China, respectively.

7.5.1.2 Immunogenicity evaluation of immunogenicity subgroup in efficacy test

The post-immunization antibody positive rate of anti-SARS-CoV-2 neutralizing antibody and RBD protein-binding antibody IgG antibody, the post-immunization antibody positive conversion rate in the pre-immunization negative population, the post-immunization antibody four-fold growth rate in the pre-immunization positive population and the pre-immunization antibody positive conversion rate in all the populations are calculated respectively in the vaccine group and placebo group in the immunogenicity subgroup in the efficacy test. The 95% confidence interval is calculated by using the Clopper-Pearson method. The difference in rates

between the vaccine and placebo groups and its 95% CI are calculated using the Miettnen-Nurminen method and the difference between the vaccine and placebo groups is statistically tested using Chi-square test /Fisher's exact probability method.

The geometric mean and bilateral 95% confidence intervals are used to statistically describe the GMT and GMT increase folds of anti-SARS-CoV-2 neutralizing antibody, RBD protein-binding antibody IgG antibody of all populations, pre-immunization negative populations and pre-immunization positive populations in the vaccine group of the placebo group in the immunogenicity subgroup, respectively. The inter-group GMT ratio of antibodies at Day 14 after full immunization and its 95% confidence interval (the vaccine group/the placebo group) are calculated, and the log-transformed group t-test is used to statistically test the differences between the vaccine group and the placebo group).

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody before immunization and at Day 14 after full course immunization of the vaccine group and the placebo group in the immunogenicity subgroup is plotted, respectively.

7.5.2 Evaluation of immune persistence

The analysis below is based on IPS.

The positive rate of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody 6 months after immunization is calculated in the vaccine group and placebo group in immunogenicity subgroup during efficacy test, respectively, Clopper-Pearson method is used to calculate its 95% confidential interval, and chi-square test /Fisher exact probability method is used for statistical test of the difference between the vaccine group and the placebo group.

The geometric mean and bilateral 95% confidence intervals are used to statistically describe the GMT and GMT increase folds of anti-SARS-CoV-2 neutralizing antibody, RBD protein-binding antibody IgG antibody of the vaccine group and placebo group at 6 months after immunization in the immunogenicity subgroup, respectively. The log-transformed group t-test is used to statistically test the inter-group differences.

The inverse distribution of antibody titer of SARS-COV-2 neutralizing antibody and RBD protein binding antibody IgG antibody at time points before and after immunization of the vaccine group and the placebo group in the immunogenicity subgroup is plotted respectively.

7.6 Safety evaluation

The analysis below is based on SS.

7.6.1 Adverse Event

Adverse events and serious adverse events are medically coded using MedDRA 24.0 or the latest version, and classified statistically at the levels of System Organ Class (SOC) and Preferred Term (PT); in addition, the solicited adverse events at the injection site (local) and non-injection site (systemic) are classified and counted according to the provisions in this protocol. Adverse events are summarized by total, regions outside China, and China, respectively.

Statistical analysis shall be performed for the Treatment Emergent Adverse Event (TEAE) that occur during the primary vaccination period in this trial, including those that occur from the first dose of vaccination to 30 days after the last dose. Adverse events occurring before vaccination and 30 days after the final dose shall be listed. If not specified, the following adverse events are TEAEs.

The number of occurrences, number of cases and incidence rate of the various adverse events below in each group are calculated respectively, and the incidence ratio and its 95% confidence interval between the two groups (experimental group/control group) are calculated by logistic regression model. Fisher's exact probability method is used to statistically compare the incidence of the adverse events above.

- ✓ All adverse events;
- ✓ Adverse events related to the investigational vaccine;
- ✓ Adverse events unrelated to the investigational vaccine;
- ✓ Adverse events with an incidence of ≥ 0.1% in any group, and adverse events related to the investigational vaccine with an incidence of ≥ 0.1% in any group;
- ✓ Adverse events with an incidence of ≥ 1% in any group, and adverse events related to the investigational vaccine with an incidence of ≥ 1% in any group;

- ✓ Adverse events with an incidence of ≥ 10% in any group, and adverse events related to the investigational vaccine with an incidence of ≥ 10 % in any group;
- ✓ Adverse events of different severity;
- ✓ Adverse events of different severity related to the investigational vaccine;
- ✓ Adverse events of different severity unrelated to the investigational vaccine;
- ✓ Adverse events with different onset times (within 30min, 0-7 days, and 7-30 days);
- ✓ Adverse events of different severity (within 30min, 0-7 days, and 7-30 days) related to the investigational vaccine;
- ✓ Adverse events of different severity (within 30min, 0-7 days, and 7-30 days) unrelated to the investigational vaccine;
- ✓ Adverse events with different doses;
- ✓ Adverse events with different doses related to the investigational vaccine;
- ✓ Adverse events with different doses unrelated to the investigational vaccine;
- ✓ Adverse events leading to withdrawal;
- ✓ Adverse events related to the investigational vaccine and leading to withdrawal

Statistically describe the relationship between the severity of adverse events and the investigational vaccine. Adverse events following each dose time of vaccination are statistically analyzed, respectively. Analysis of adverse events following each dose time shall be performed on the safety set of each dose time.

Forest plots comparing the incidence of adverse events between the two groups shall be presented by vaccine group top 10 preferred terms.

List a list of adverse events related to the investigational vaccine, a list of the adverse events unrelated to the investigational vaccine, a list of adverse events that occurred during the non-vaccination period, and a list of adverse events of Grade 3 severity and above.

7.6.2 Serious Adverse Events (SAE)

The serious adverse events (SAEs) that occurred after the first dose are statistically analyzed, including those occurring within 12 months from the first dose vaccination to the full vaccination. Serious adverse events shall be summarized by total, regions outside China, and in China, respectively. If not specified, the following serious adverse events are TESAEs.

The number of occurrence, number of cases and incidence of the various serious adverse

events of each group below are calculated, and the inter-differences shall be statistically compared using Fisher's exact probability method.

- All serious adverse events
- Serious adverse events related to the investigational vaccine;
- Serious adverse events unrelated to the investigational vaccine;
- Serious adverse events leading to death;
- Serious adverse events related to the investigational vaccine that lead to death.

Make a list of serious adverse events.

7.7 Subgroup analysis

- **Efficacy and safety analysis for the following subgroups shall be conducted:**
  - ✧ Age group (18-59 years vs. 60 years and above)
  - ✧ Race (Asian population vs Chinese population)
- **Efficacy for the following subgroups shall be analyzed:**
  - ✧ Different participating countries (including Uzbekistan, Indonesia, Pakistan and Ecuador)
  - ✧ Combined diseases (including top 5 diseases and obesity)
- **Immunogenicity for the following subgroups shall be analyzed:**
  - ✧ Age group (18-59 years vs. 60 years and above)

7.8 Interim analysis

This is a case-driven study. If 52 cases of COVID-19 (1/3), 104 cases of COVID-19(2/3) are observed during the study course, the interim analysis shall be conducted. Total error of type I shall be controlled through the consumption function of O'Brien-Fleming.

- If 52 cases of COVID-19 with any severity are collected for the first interim analysis, the nominal test level α_1=0.0001 (single-sided) of IA shall be calculated according to O'Brien-Fleming consumption function;
- If no valid conclusion can be obtained in the first interim analysis, the second interim analysis shall be conducted when 104 COVID-19 cases are collected, and the nominal test level of this interim analysis is α_2=0.0060 (single-sided);
- If no valid conclusion can be obtained in the second interim analysis, the final analysis shall be conducted when 156 cases of COVID-19 are observed, and the nominal test

level for FA is $\alpha_3 = 0.0231$ (single-sided).

During the study course, nominal significance level of IA and FA is estimated through the consumption function of O'Brien-Fleming according to the actual number of observed cases of COVID-19 at IA. If there are more than planned cases of COVID-19 at IA, α is assigned again through the method of O'Brien-Fleming. If interim analysis is not performed in actual trial, no adjustment shall be made for category I errors and a two-sided α=0.05 shall be used for statistical analysis.

7.9 Multiplicity issue

In the efficacy analysis, O'Brien-Fleming method shall be used to allocate α in interim analysis (see chapter 7.8 for details). After the efficacy criteria of the test protection efficacy evaluation is met, the noninferior bridging comparison shall be conducted for immunogenicity in China and regions outside China.

In the immunogenicity evaluation, the anti-SARS-CoV-2 neutralizing antibody GMT 14 days after full immunization in the pre-immunization negative population of the vaccine group in China is required to be noninferior to that of the regions outside China to bridge the vaccine's efficacy outside China to China. Therefore, no Class I error correction is required.

In the safety evaluation results, the calculated P value is only the nominal P value, which is mainly used to describe the strength of the association between the evaluation endpoint and the treatment subgroup, and is not used as the basis for formal statistical inference.

7.10 Handling of dropouts or missing data

In the efficacy evaluation, the incidence rate and vaccine efficacy rate shall be calculated using the actual exposure time of subjects as the denominator and the observed cases during the exposure time as the numerator. If the subjects do not conduct a follow-up visit, the protection efficacy of vaccine shall be evaluated based on non-morbidity for this visit.

In the statistical analysis for the Full Analysis Set for Immunogenicity, for those with post-immunization serum neutralizing antibody test results missing, the Last Observation Carried Forward (LOCF) method shall be used to fill in the data and further derive and calculate the corresponding immunogenicity endpoint.

Missing data from other immunogenicity endpoint and safety endpoint are not processed in this study.

Version History

EDITION	Version time	Writer	Update content
1.0	August 02, 2021	Tian Ye	Initial version