Project title:	**Phase II clinical trail for SARS-CoV-2 Vaccine (Vero cells), Inactivated**
Protocol title:	**A randomized, double-blind, placebo-controlled clinical trial (phase II) to evaluate the safety and immunogenicity of the SARS-CoV-2 vaccine (Vero cells), inactivated in healthy adults aged 18 and above**

IP: SARS-CoV-2 Vaccine (Vero cells), Inactivated
Registration classification: Preventive Biological Product Class 1.1
Project No.: JSVCT101
Protocol No.: 2020L001-1B
Version No.: 1.2
Date of version: 30 September, 2020
Authorized signatory of the protocol:_____
(Signature)
Date of signing:_____DD/MM/YYYY

Sponsors: Shenzhen Kangtai Biological Products Co., Ltd.
Beijing Minhai Biotechnology Co., Ltd.

Statement of principal investigator

I agree to:

- ✧ Assume the responsibilities of the principal investigator for the clinical study at this research institution.
- ✧ Ensure that the study can be conducted in accordance with the trial protocol, the revised protocol agreed by all parties, and other clinical study standard operating procedures of Shenzhen Kangtai Biological Products Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd..
- ✧ Ensure that our personnel involved in the study have the information provided by the sponsors on the experimental vaccine and know the duties and responsibilities related to the study as stated in the protocol.
- ✧ Ensure that no changes will be made to the protocol without review or written approval of the sponsors and the ethics committee, unless otherwise required to eliminate immediate harm to the subject or to comply with the requirements of the pharmacovigilance authorities (e.g. issues involved in administrative management).
- ✧ I have full knowledge of the proper use of the experimental vaccine as described in the protocol and full knowledge of other information provided by the sponsors, including but not limited to the current investigator's brochure or its equivalents.
- ✧ I know and will comply with the Good Clinical Practice (GCP) and all relevant regulatory requirements for the vaccine clinical trial.

Study title	Clinical Trial (Phase II) of SARS-CoV-2 Vaccine (Vero cells), Inactivated
Detailed description of the study title	This randomized, double-blind, placebo-controlled clinical trial (phase II) aims to evaluate the safety and immunogenicity of SARS-CoV-2 Vaccine (Vero cells), Inactivated in healthy adults aged 18 and above.
Study vaccine	SARS-CoV-2 Vaccine (Vero cells), Inactivated
Project No.	JSVCT094
Protocol No.	2020L001-1B
Date of protocol	September 30, 2020
Version No.	1.2
Investigator	Name: Pan Hongxing Title: Chief Physician Affiliation: Jiangsu Provincial Center for Disease Control and Prevention (Jiangsu Provincial Public Health Research Institute) Address: 172 Jiangsu Road, Nanjing PC: 210009 Tel: 025 -83759903 Fax: 025-83759911 E-mail：panhongxing@126.com
Investigator (Signature)	Date of signing: DD/MM/YYYY

History of protocol revision

No.	Original version number/version date/revision	Current version number/version date/revision description
1	V1.1/29-SEP-2020/"8.10 Study suspension and early termination"	V1.2/30-SEP-2020/adding the information on processing after the trial suspension.
2	V1.1/29-SEP-2020/"9.4 Exclusion criteria for subsequent vaccinations"	V1.2/30-SEP-2020/adding the information on the treatment and exclusion of confirmed COVID-19 cases during the trial.
3	Upon V1.2 of the protocol came into effect, V1.1 was abolished.	
4	V1.0/23-SEP-2020/Sponsor: "Shenzhen Kangtai Biological Products Co., Ltd."	V1.1/29-SEP-2020/revised to: "Shenzhen Kangtai Biological Products Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd."
5	V1.0/23-SEP-2020/project title: "Phase I/II Clinical Trial for SARS-CoV-2 vaccine (Vero cells), inactivated"	Revised in protocol version 1.1 /20200929/ as: "Clinical Trial (Phase II) of SARS-CoV-2 vaccine (Vero cells), inactivated "
6	V1.0/23-SEP-2020/protocol title: "A Phase I/II randomized, double-blind, placebo-controlled clinical trial to evaluate the safety and immunogenicity of SARS-CoV-2 vaccine (Vero cells), inactivated in healthy adults aged 18 years and above"	Revised in protocol version 1.1 /20200929/ as: "a randomized, double-blind, placebo-controlled clinical trial (phase II) to evaluate the safety and immunogenicity of SARS-CoV-2 vaccine (Vero cells), inactivated in healthy adults aged 18 and above"
7	V1.0/23-SEP-2020/8.6.1 Experimental vaccine: "The protein content should not be less than 5 μg", "The protein content should not be less than 10 μg"	V1.1/29-SEP-2020/revised to: "Protein content is 5 μg", "Protein content is 10 μg"
8	Originally in protocol version 1.0 /20200923/: 8.7.1 Primary Endpoint – "the positive seroconversion rate of neutralizing antibody and IgG antibody (ELISA method) against SARS-CoV-2 virus 28 days after the whole immunization"	Revised in protocol version 1.1 /20200929/ as: – "the positive seroconversion rate and antibody level of neutralizing antibody against SARS-CoV-2 virus 28 days after the whole immunization" – "the positive seroconversion rate of IgG antibody (ELISA method) against SARS-CoV-2 virus 28 days after the whole immunization"
9	V1.0/23-SEP-2020/8.10 Study suspension and early termination "Criteria for suspension: – The trial will be suspended in the event of one or more Grade 4 or serious adverse events that may be related to vaccination during the study period; – When more than 15% of subjects have experienced adverse events of grade 3 and above, including local reactions, systemic reactions, vital signs and abnormal laboratory data, the investigators, sponsors and the data security monitoring committee will discuss together to decide whether to suspend the trial. Criteria for early termination: – The trial will be terminated early when there is more than 1 death that may be related to vaccination during the study; – When more than 30% of subjects have experienced adverse events of Grade 3 and above, including local reactions, systemic reactions, vital signs and abnormal laboratory data, the investigators, sponsors and the data security monitoring committee will discuss together to decide whether to terminate the trial early;	V1.1/29-SEP-2020/revised to: Criteria for suspension: – The trial will be suspended in the event of 1 grade 4 or serious adverse event that may be related to vaccination during the study period; – The trial will be suspended when more than 15% of subjects have experienced adverse events of grade 3 or above. Criteria for early termination: – The trial will be terminated early when there is 1 death that may be related to vaccination during the study period; – When more than 30% of subjects have adverse events of Grade 3 and above, the investigators, sponsors and the data security monitoring committee will discuss together to decide whether to terminate the trial early;
10	V1.0/23-SEP-2020/10.4.8 Medical treatment of adverse events: It should be recorded as an adverse event and reported to the ethics committee. The investigators should continue to follow up until 12 months after the birth of the infant to complete the follow-up of the physical conditions of the subject and infant."	V1.1/29-SEP-2020/revised to: "It should be recorded as an adverse event. The investigator should continue to follow up the physical conditions of the subject and the infant until 12 months after the birth."

11	V1.0/23-SEP-2020/10.5.2 Blood sample processing and storage: "The venous blood collected for immunogenicity testing should be centrifuged to separate serum in time on the same day. The serum should be put in 3 tubes, 0.5 ml/tube at least, and they should be frozen at -20°C for unified test. Sera in tube 1 (Set A) and tube 2 (Set B) are used for antibody detection. Serum in tube 3 (Set C) of phase II trial is available as a reserve sample.	V1.1/29-SEP-2020/revised to: "The venous blood collected for immunogenicity testing should be centrifuged to separate serum in time on the same day. The serum should be put in 4 tubes, 0.5 ml/tube at least, and they should be frozen at -20°C for unified test. Sera in tube 1 (Set A) and tube 2 (Set B) are used for antibody detection. Sera in tube 3 (Set C) and tube 4 (Set D) are available as reserve samples.
12	V1.0/23-SEP-2020/10.7.5 Interim analysis: "The trial is scheduled for a safety interim analysis at the following time points: ➢ 28 days after the whole immunization of the last subject in phase II trial. Independent statisticians from DSMB will issue an unblinded safety report regarding the above-mentioned mid-term analysis. The safety evaluation will be carried out by DSMB.	Revised in protocol version 1.1 /20200929/ as: "At present there's no plan to conduct a mid-term safety analysis. If such an analysis does take place later, independent statisticians from DSMB will issue an unblinded safety report accordingly. The safety evaluation will be carried out by DSMB."

Protocol summary

Study title	Clinical Trial (Phase II) of SARS-CoV-2 Vaccine (Vero cells), Inactivated
Detailed description of the study title	The study is a randomized, double-blind, placebo-controlled clinical trial conducted to evaluate the safety and immunogenicity of SARS-CoV-2 Vaccine (Vero cells), Inactivated in healthy adults aged 18 and above.
Trial objective	To evaluate the safety and immunogenicity of SARS-CoV-2 Vaccine (Vero cells), Inactivated in healthy people aged 18 and above
Disease prevention	To prevent the diseases caused by SARS-CoV-2.
Subjects	Healthy adults aged 18 and above.
Sample size	Total of 1000 subjects taking part in this phase II trial, among which 750 aged from 18-59 (including 250 in the 0-28-56 d immunization group, 250 in the 0-28 d group, and 250 in the 0-14 d group.) Another 250 subjects aged 60 and above take part in the 0-28-56 d immunization group.
Randomization and blindness	Randomization and blindness are achieved by vaccine blinding, and the randomization list is generated by means of SAS. In each of the immunization groups (no matter from 18-59-year-old group or ≥60-year-old group), the subjects are allocated at a ratio of 2:2:1 into medium-dose group, high-dose group and placebo group, respectively. Subjects passing the screening are assigned study numbers in descending order of enrollment, and receive the correspondingly numbered experimental vaccine.
Experimental vaccine	experimentalSARS-CoV-2 Vaccine (Vero cells), Inactivated and the placebo were jointly developed by Shenzhen Kangtai Biological Products Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd., and have passed the inspection of the National Institutes for Food and Drug Control. Experimental vaccines: Medium-dose vaccine: in a liquid dosage form, 0.5 ml/dose, containing 5 μg of SARS-CoV-2 protein. High-dose vaccine: in a liquid dosage form, 0.5 ml/dose, containing 10 μg of SARS-CoV-2 protein. Placebo control: In a liquid dosage form, 0.5 ml/dose, containing 0.25 mg of aluminum hydroxide adjuvant. Vaccination site and route: At the deltoid muscle of the outer upper arm, intramuscularly. Storage and transport conditions: Store and transport at 2-8°C out of the sun, and prevent from freezing.
Study endpoints	Primary endpoints: − "the positive seroconversion rate and antibody level of neutralizing antibody against SARS-CoV-2 virus 28 days after the whole immunization" − The positive seroconversion rate of IgG antibody (ELISA method) against SARS-CoV-2 virus 28 days after the whole immunization Secondary endpoints: − Incidence of adverse events within 28 days after each dose of vaccination. − Incidence of serious adverse events from the first dose to 12 months after whole immunization. − The antibody level of neutralizing antibody and IgG antibody (ELISA method) against SARS-CoV-2 virus 28 days after the whole immunization − Positive seroconversion rate and antibody level of neutralizing antibody and IgG antibody (ELISA method) against SARS-CoV-2 virus 28 days after the 2nd dose (as for 3-dose immunization group) or 14 days after the whole immunization (as for 2-dose immunization group)

	− Positive seroconversion rates and the levels of SARS-CoV-2 neutralizing antibodies and IgG antibodies (ELISA method) at 3, 6 and 12 months after full-course immunization.
Study plan	For each age group, the phase II trial is only permitted, when safety and tolerability have been preliminary confirmed in the phase I trial 7 days after the 1st dose of the high-dose group. Volunteers will be screened from the resident healthy adults aged 18 and above in accordance with the principles of voluntary registration, informed consent, and compliance with inclusion and exclusion criteria. When enrolled, every subject will be given a code number and get the correspondingly vaccine. Subjects in the 18-59-year-old group will receive the experimental vaccine or placebo according to the 2-dose 0-14 d or 2-dose 0-28 d immunization schedule, or 3-dose 0-28-56 d immunization schedule, respectively. Subjects in the ≥60-year-old group will receive the experimantal vaccine or placebo according to the 3-dose 0-28-56 d immunization schedule. After each dose, the subjects need to stay on site for 30 min to see if there's any immediate response. During the following 7-day systematic follow-up, the subjects should record their daily observation results on the Diary Card. After each dose, subjects will receive a telephone visit on day 3 and an intensive visit on day 7 by the researchers, to verify the safety of the vaccination. Subjects should carry out safety observations on their own from day 8 to day 28 after each vaccination. After each dose, subjects will receive a telephone visit on day 14 and an intensive visit on day 28 by the researchers, to verify the safety of the vaccination. About 5 ml of venous blood will be collected from subjects in the 2-dose immunization group before the 1st dose, 14 days, and 28 days after whole immunization, and fro subjects in the 3-dose immunization group, before the 1st and 3rd dose and 28 days after whole immunization, respectively, in order to detect the serum antibody titers. Subjects will be followed up passively during a 12-month long-term safety observation after whole immunization to verify the incidence of serious adverse events during the study period. A visit (face-to-face/telephone visit) will be conducted once each at 3, 6, 9 and 12 months after full-course immunization to collect the data on the incidence of SAEs. To observe the immune persistence in subjects who have completed the full-course immunization, about 5 ml of venous blood will be collected at 3 months (+30 d), 6 months (+30 d) and 12 months (+30 d) after the full-course immunization, and the serum will be separated for antibody detection and immune persistence evaluation.
Study period	For each subject it will last about 15 months from enrollment to discharge at the end of the final visit, and some subjects may withdraw at some point during the study. This study will take about 16 months from the time the first subject is enrolled to the time the last subject is discharged at the end of the final visit.
Criteria for suspension and early termination	Criteria for suspension: − The trial will be suspended if a Grade 4 adverse event or serious adverse event that may be related to vaccination during the study period; − The trial will be suspended when more than 15% of subjects have experienced adverse events of Grade 3 or above. Criteria for early termination: − The trial will be terminated early when there is 1 death that may be related to vaccination during the study; − When more than 30% of subjects have adverse events of Grade 3 and above, the investigators, sponsors and the data security monitoring committee will discuss together to decide whether to terminate the trial early; − The sponsor requests full termination of the trial with justification; − The administrative authorities request full termination of the trial with justification. − The ethics committee requests full termination of the trial with justification.

Criteria for inclusion:	– Resident healthy adults aged 18 and above; – Volunteers give their consent and sign an informed consent form; – Volunteers comply with the requirements of the clinical trial protocol. – Those with axillary temperature ≤ 37.0°C. – Female subjects who are not pregnant and breastfeeding, without childbearing potential within the first 3 months of enrollment can be enrolled. And they should take effective contraceptive measures within 2 weeks prior to enrollment.
Criteria for exclusion	– Those subjects who have traveled abroad or have been to a village/community where an outbreak has occurred within 14 days before vaccination, have been exposed to COVID-19 infected or suspected cases, or are in quarantine, or come from their village/community where there are COVID-19 infected or suspected cases; – Confirmed or suspected cases, or asymptomatic infections of COVID-19 (check the China Disease Prevention and Control Information System); – Self-reported history of SARS virus infection; – Positive RT-PCR test results on pharyngeal swabs; – Those with a self-reported history of SARS virus infection; – Those with previous severe allergic reactions to vaccination (e.g., acute allergic reactions, urticaria, skin eczema, dyspnea, angioneurotic edema, or abdominal pain) or allergic to known components of the SARS-CoV-2 Vaccine (Vero cells), Inactivated; – Those with a history or family history of convulsions, epilepsy, encephalopathy or mental illness; – Those with congenital malformations or developmental disorders, genetic defects, or severe malnutrition, etc.; – Those known or suspected to have diseases including: severe respiratory disease, severe cardiovascular disease, severe liver or kidney disease, medically uncontrollable hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg; systolic blood pressure ≥ 150 mmHg and diastolic blood pressure ≥ 100 mmHg for subjects aged ≥ 60 years), complications of diabetes mellitus, malignancy, various acute diseases or acute episodes of chronic disease; – Those diagnosed with congenital or acquired immunodeficiency, HIV infection, lymphoma, leukemia or other autoimmune diseases; – Those with a history of coagulation abnormalities (such as deficiency of coagulation factors, coagulopathies); – Those on anti-tuberculosis treatment; – Those who have received other experimental drugs within 6 months prior to vaccination; – Those who have received immune enhancement or inhibitor therapy within 3 months before vaccination (continuous oral or instillation for more than 14 d); – Those who have received blood products within 3 months before vaccination; – Those who have received a live attenuated vaccine within 14 days before vaccination; – Those who have received other vaccines within 7 d before vaccination; – Those with other conditions not eligible for participating in the study at the investigator's discretion.
Statistical analysis	Initial analysis: At the end of the visit and immunogenicity analysing at day 28 after the last subject completes the final visit, subjects can be unblinded for statistical analysis when all parties have jointly reviewed and locked the database. Updated analysis: Long-term safety observation and statistical analysis of immune persistence will be performed after completion of SAE observation at 12 months after full-course immunization and completion of antibody detection for immune

	persistence observation at 3, 6 and 12 months after full-course immunization.

Glossary & Abbreviation

GCP	Good Clinical Practice
NMPA	National Medical Products Administration
CDC	Center for Disease Control and Prevention
EC	Ethics Committee
PI	Principal Investigator
SOP	Standard Operation Procedure
CRF	Case Report Form
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GMT	Geometric Mean Titer
AE	Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
SAE	Serious Adverse Event
ITT	Intention-To-Treat
SS	Safety Set
FAS	Full Analysis Set
PPS	Per Protocol Set

Table of Contents

Clinical Trial Protocol (Phase II) of SARS-CoV-2 Vaccine (Vero cells), Inactivated

1. Purpose and introduction

SARS-CoV-2 Vaccine (Vero cells), Inactivated jointly developed by Shenzhen Kangtai Biological Products Co.,Ltd. and Beijing Minhai Biotechnology Co., Ltd. is used to prevent diseases caused by SARS-CoV-2. To evaluate the safety and immunogenicity of the SARS-CoV-2 Vaccine (Vero cells), Inactivated in healthy adults aged 18 and above. This plan is formulated according to the requirements of the clinical trial approval document of the National Medical Products Administration, and in accordance with the requirements of the *Vaccine Management Law*, the *Drug Registration Regulation*, the *Good Clinical Practice* (GCP) and the *Technical Guiding Principles for Clinical Trials of Vaccines*.

2. Trial site

The clinical trial site is located in Huaiyin District, Huai'an City, Jiangsu Province.

3. Clinical trial participants

3.1. Organization responsible for the clinical trials

The clinical trial was conducted by Jiangsu Center for Disease Control and Prevention, whose main responsibilities are:

- Participate in the formulation of vaccine clinical trial plan and forms and cards required for the trial;
- Participate in drafting informed consent for vaccination, drafting SOP for clinical trial site operation, applying for approval from the ethics committee, and applying for the record of the vaccine clinical trial institution at the trial site;
- Organize and implement clinical trials, organize and implement the selection of clinical trial observation points that meet the requirements of GCP, and control the quality of clinical trial implementation process;
- Responsible for reporting serious adverse reactions/events occurred during the clinical trials to the sponsor in a timely manner, conducting investigation and handling the events, and reporting suspicious and unexpected serious adverse reactions provided by the sponsor to the ethics committee;
- Participate in database locking and save the locked database backup for verification;
- Responsible for writing clinical trial summary report.

3.2 Clinical research institute

The clinical research site unit is Jiangsu Center for Disease Control and Prevention, whose main responsibilities are:

- Providing a suitable test site and cooperate to complete the filing of vaccine clinical trial institutions at the trial site;
- Organize personnel with corresponding professional skills and rich clinical research experience to participate in the work of the research site. Organize all participating researchers to read and understand the contents of the research plan in detail, and strictly follow the plan to ensure that there is sufficient time to complete the clinical trial within the time limit specified in the plan;
- Organize on-site implementation, including the organization and selection of subjects, obtaining the informed consent signed by the subjects, enrollment, vaccination, follow-up, collection, processing, freezing and inspection of biological samples;
- Responsible for data-entry, ensure all data collected are true, accurate, complete and legal;
- Accept the monitoring and audit of the CRA or inspectors dispatched by Party A and the inspection of the vaccine administration to ensure the quality of the clinical study.
- Ensure that the subjects are properly treated in case of adverse reactions / events during the study. In case of serious adverse reactions / events, take appropriate treatment measures to the subjects immediately, report to the sponsor, and report to the ethics committee the suspicious and unexpected serious adverse reactions provided by the sponsor;

- Responsible for the preservation of clinical trial data during clinical trials.

3.3 Sponsors

The sponsors of the clinical trial are Shenzhen Kangtai Biological Products Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd. Their main responsibilities are:

- Provide the preliminary plan of clinical trial, sign and seal the final plan;
- Provide on-site application documents such asclinical trial approval documents, clinical trial researcher's Manual (preclinical safety information), product implementation standards, etc.;
- Responsible for providing sufficient vaccines for clinical research, and issue qualified inspection report;
- Responsible for appointing inspectors to assess and approve the clinical trial site, fulfilling the supervision duties according to the requirements of GCP, and bearing the final responsibility for the quality of clinical trials;
- Participate in the investigation and treatment of vaccine abnormal reaction cases, and be responsible for providing medical treatment and relevant compensation for the abnormal reaction cases clinically proved to be related to vaccination according to relevant regulations;
- Responsible for providing funds for the clinical research;
- Timely conduct scientific research and judgment on the serious adverse reactions / events reported by researchers, and deal with them as required.

3.4 Clinical trial monitoring institute

The clinical trial monitoring unit is Beijing Minhai Biotechnology Co., Ltd. It's main responsibilities are:

- Formulate corresponding monitoring plan;
- Monitor the researcher's implementation of the trial plan during the trial, and confirm that the informed consent form of all subjects is obtained before the trial;
- Get to know the selection rate of subjects and the progress of the trial, and confirm that the selected subjects are qualified;
- Confirm that all data records and reports of vaccination, safety follow-up, and on-site data are correct and complete;
- Confirm that all adverse events are recorded, and that serious adverse events are reported and recorded within the specified time;
- Verify that the trial vaccine is supplied, stored, distributed, and recovered in accordance with relevant laws and regulations;
- Verify that all specimens collected in the trial are packed and preserved according to the trial plan;
- Make corresponding monitoring records, and form progress and final monitoring report.

3.5 Clinical trial data management institute

The clinical trial data management units are Xi'an Riehen Life Science Technology Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd. whose main responsibilities are:

- Design eCRF, establish database, control user rights, draft *eCRF Completion Guidelines*, *Note CRF*, *Data Management Plan*, Data Verification Plan, etc according to the requirements of relevant national regulations and the clinical trial plan;
- Coordinating sponsors, researchers, clinical research associates, statisticians, etc., to conduct EDC system user training and user acceptance testing (UAT);
- After the EDC system goes on-line, conduct data verification, issue query and close query, carry out external data management, data audit, database locking, data export and transmission, data backup and recovery, data and data management document archiving, etc., and finally complete the *Data Management Report*.

3.6 Test specimen testing institute

The clinical trial specimen testing units are National Institutes for Food and Drug Control (NIFDC) and Chinese Center For Disease Control And Prevention (CCDC). Their main responsibilities are: NIFDC is responsible for detecting the concentration of neutralizing antibody of pseudovirus and IgG antibody. CCDC is responsible for testing the neutralizating antibody titer of SARS-CoV-2.

3.7 Statistical analysing institute

The **institut** responsible for statistical analysis of the clinical trial is Beijing Key Tech Statistical Technology Co., Ltd., which is mainly responsible for:

- Statistical design of the plan;
- Randomization and blinding;
- Statistical analysis plan and statistical analysis report preparing;
- Statistical programming and statistical analysis;
- Preparation of analytical database to be reported;
- Review of statistics related documents such as clinical trial plan, CRF, data verification resolution, clinical research report, etc.

4. Background

4.1 Background of the disease

COVID-19 is a kind of pneumonitis caused bySARS-CoV-2. As a β-type coronavirus, single-stranded positive scene RNA virus, SARS-CoV-2 is enveloped, and particles are round or elliptic, often pleomorphic, with diameters of 60~140nm. The full genome length is about 29,800 basic groups. It involves 14 open coding frames that encodes 27 viral proteins [1]. People who are infected by SARS-CoV-2 are the major infection source and SARS-CoV-2 has the characteristic of human-to-human transmission. The infected person without symptoms also can be the infection source. Respiratory droplets and close contact are the main route of transmission. Aerosol transmission is also the possible way if the infected person is exposed to high-concentration aerosols for a long time in a relatively closed environment. SARS-CoV-2 can be separated from faeces and urine [2]. Fever, cough, dyspnoea and muscle pains are the most common symptoms of COVID-19. The incubation period of SARS-CoV-2 ranges from 1d to 14d, averaging at about 10d. SARS-CoV-2 is infectious during the incubation period, which is significantly different from SARS. Some study reported that 30%-60% of patients infected by SARS-CoV-2 have no symptoms or only mild symptoms, but they still have strong ability to spread the virus. These recessive infected persons might cause a new epidemic [3]. The basic reproduction number (R0) of SARS-CoV-2 ranges between 2.2 and 5.7 [4,5]. All people are susceptible to COVID-19 [6]. There's no specific medicine for COVID-19, but it can only be controlled according to the principle of early diagnosis, early quarantine and early ICU as much as possible. Basic therapy for pneumonia, antiviral drugs and anti-inflammatory combined with oxygen supply are applied as treatment of COVID-19. Suspected and diagnosed cases are quarantined at designated places. Critical cases are admitted to ICU as early as possible.

On March 11, 2020, WHO announced that COVID-19 epidemic has brought a "global epidemic". In China, COVID-19 is included into the List of Category B Infectious Diseases regulated by the *Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases*.

4.2 Research progresses of OVID-19 vaccine

Now, COVID-19 caused by SARS-CoV-2 has spreaded to more than 210 countries and regions. With the development of the COVID-19 epidemic, all countries in the world are devoted to research and development (R&D) of COVID-19 drugs and vaccines independently or cooperatively. At present, there are more than 120 candidate COVID-19 vaccines under development in the world. China is promoting vaccine R&D from five technical routes simultaneously, including inactivated vaccine, recombination genetic engineering vaccine, adenovirus vector vaccine, nucleic acid vaccine and attenuated influenza viral vector vaccine. Among them, the adenovirus vector vaccine achieved the quickest progress and it was the first COVID-19 vaccine which started the phase-II clinical study in the world.

The technical route for R&D of inactivated vaccine is the most mature, manifested by the explicit evaluation method and easy connection to scaled production process and easy transformation into multivalent vaccine. Since April, 2020, the National Medical Products Administration has approved clinical trails of COVID-19 inactivated vaccines which were developed by Wuhan Research Institute of Biological Products of China Biotechnology, Beijing Kexing Zhongwei Biotech Co., Ltd, Beijing Research Institute of Biological Products of China Biotechnology, and Institute of Medical Biology, Chinese Academy of Medical Sciences. Now, all of these vaccines have entered into the phase-III clinical tests. Emerging infectious diseases usually are characteristics of fast spreading, high lethality and lack of specific preventive and therapeutic drugs. Vaccine is the most economic and effective mean to control and prevent infectious diseases. With the spreading of COVID-19 epidemic, the importance of R&D of vaccine is further highlighted.

5. Preclinical Study of vaccines

5.1 Studies on animal immunogenicity

In the cynomolgus monkey animal model, one immunization of hindquarter muscle was performed to the cynomolgus monkey at 0d, 7d, 21d and 35d, respectively. The dosages were set 0.5ml per immunization for the 5μg dosage group (5μg/0.5ml), 0.5ml per immunization for the 5μg dosage group (10μg/0.5ml) and 2.0ml per immunization for the 40μg dosage group (10μg/0.5ml). Moreover, vein blood samples were collected from the hindquarters before the immunization and at 7d, 14d, 21d, 35d and 49d of the first immunization, from which serum was separated to detect the neutralizing antibody titer. According to test results, the cynomolgus monkey began to generate neutralizing antibodies since 7d after the immunization. The neutralizing antibody titer of serum increased gradually with the increase of immunization time and dosage, showing evident dose-effect relations. At 14d after the first immunization, the neutralizing antibody titer (GMT) of cynomolgus monkey serum in the 5μg, 10μg and 40μg dosage groups were 61, 67 and 197. At 21d after the first immunization, GMT of the 5μg and10μg dosage groups declined slightly to 33 and 50, while the GMT of the 40μg dosage group increased to 279. After the booster immunization, GMT of the 5μg, 10μg and 40μg dosage groups increased significantly to 437, 394 and 1194 at 35d, and then to 1040, 905 and 1874 at 49d. To sum up, high level of neutralizing antibodies are generated at different time points after 2 weeks of immunization to the cynomolgus monkey by different dosages of COVID-19 inactivated vaccines. This proves the good immunogenicity of the COVID-19 inactivated vaccine to cynomolgus monkey.

In the rats animal model, one immunization of hindquarter muscle was performed to the rats at 0d, 7d, 21d and 35d, respectively. The dosages were set 0.5ml per immunization for the 5μg dosage group (5μg/0.5ml), 0.5ml per immunization for the 5μg dosage group (10μg/0.5ml) and 1.5ml per immunization for the 30μg dosage group (10μg/0.5ml). Moreover, vein blood samples were collected from the neck before the immunization and at 7d, 21d, 35d and 49d of the first immunization, from which serum was separated to test the neutralizing antibody titer. According to test results, the rats began to generate neutralizing antibodies since 7d after the immunization. The serum neutralizing antibody increased gradually with the increase of immunization time and dosage. GMT was 905～1114 at 21d, 2307～2941 at the 35d and 3620～5120 at 49d.after the first immunization. From the first immunization to the 35d, there's no evident differences among three dosage groups in term of the average GMT. Moreover, GMT of the 5μg dosage group and the 30μg dosage group was higher than that of the 10μg dosage group at 49d. In a word, a high level of neutralizing antibodies is generated in rats after immunization with different dosages of COVID-19 inactivated vaccines. This proves the good immunogenicity of the COVID-19 inactivated vaccine to rats.

5.2 Studies on animal protective results

A total of 12 rhesuses were divided evenly into the high-dose group (n=4), low-dose group (n=4) and the model group (n=4) randomly. The dosages to the high-dose group and low-dose group were 10μg/0.5ml and 5μg/0.5ml. Each rhesus was injected with 0.5ml of the COVID-19 inactivated vaccine. The immunization schedule covered the 0d and 14d immunizations. Neutralizing antibodies were tested at 21d after the immunization and a challenge experiment was performed by the dosage of 10^6TCID_{50}/pc at 22+2d after the immunization. During the experiment, ordinary symptoms of animals were observed and recorded every day. Throat swabs were collected at 3d, 5d and 7d after the a challenge experiment to test the viral loads.

According to test results, body temperature of the model group was not increased significantly. At 7d after the challenge experiment, the viral load of the throat swabs was $10^{5.83}$copies/ml and the total mean of viral load was $10^{6.50}$copies/ml, accompanied with moderate (1/4) ~severe (3/4) interstitial pneumonitis. The high-dose group didn't show abnormal body temperature and the viral load of throat swab at 7d after the challenge experiment was tested negative in comparison with the model group. The viral load of lung tissues decreased by 3.72lg and three rhesuses were detected negative. There were 4 cases of mild interstitial pneumonitis. The low-dose group didn't show abnormal body temperature and the viral load of throat swab at 7d after the challenge experiment was$10^{4.15}$copies/ml in comparison with the model group, including 2 negative cases. Moreover, all cases were tested negative for viral load in lung tissues. There were 4 cases of mild interstitial pneumonitis. Results prove that high dose and low dose of COVID-19 inactivated vaccine (Vero cells) can protect the infected rhesuses significantly. A GMT higher than 1:32 can inhibit the SARS-CoV-2 effectively and relieve lesions of pneumonia.

5.3 Safety Assessment of animals

Muscle single-dose toxicity test of RatsResults showed that Sprague-Dawley was provided with single muscle injection of SARS-CoV-2 Vaccine (Vero cells), Inactivated at 4 doses/pc (40μg/pc), showing no deaths or moribundity. Hence, the maximum tolerance dose (MTD) is higher than or equal to 40μg/pc.

Muscle repeat-dose toxicity test of rats

Results showed that all dose groups presented transient growths of Neut and FIB and a reduction of Lymph after repeat muscle rejections of COVID-19 inactivated vaccine (Vero cells) to rats every week or every 2 weeks at the doses of 5, 10 or 30 μg/pc for 7 weeks, totally 5 injections. At the end of immunization, Glb and AST increased, while Alb and A/G decreased. All these four parameters recover to the normal levels at the end of recovery period. Irritant and inflammatory reactions which might be related with aluminum adjuvant could be seen on outer membrane of ischiadic nerve and skins (surrounding the mammary gland) of local injection group and the 30 μg/pc group, while no evident systematic toxic reactions were observed. Certain levels of anti-S proteins IgG antibodies and neutralizing antibodies can be detected from animals, but no immunotoxicity reactions have been seen. According to current data, the No Observed Adverse Effect Level (NOAEL) was 30 μg/pc.

Muscle repeat-dose toxicity test of cynomolgus monkey

According to test results, irritant reactions (mild or moderate granulomatous inflammation) related with test samples can be seen after local injection of SARS-CoV-2 Vaccine (Vero cells), Inactivatedevery week or every 2 weeks for 7 successive weeks (total 5 injections) under the dosage of 5, 10 and 40 μg/pc. No systematic toxic reactions were observed. Certain levels of anti-S proteins IgG antibodies and neutralizing antibodies can be detected from animals, but no immunotoxicity reactions have been seen. Therefore, the NOAEL was 40 μg/pc.

According to the systematic positive allergic reaction test results of guinea pigs, three successive sensitizations after muscle injection of SARS-CoV-2 Vaccine (Vero cells), Inactivatedevery two days at the dosages of 0.1 dose/pc and 1 dose/pc will cause allergies. Intravenous injections at the dosages of 0.2 dose/pc and 2 doses/pc were performed at 14d and 21d of the last sensitization, but the guinea pigs didn't show any systematic positive allergic reaction.

6. Product features

SARS-CoV-2 Vaccine (Vero cells), Inactivated is manufactured by inoculating novel coronavirus onto Vero cells and through viral culture, harvest, inactivation, purification and adjuvant adsorption. It is used to prevent COVID-19. SARS-CoV-2 Vaccine (Vero cells), Inactivated is a uniform milky white suspension after shaking, without clot or foreign matters. It contains sARS-CoV-2 (inactivated) antigens, $Al(OH)_3$, NaCl, phosphate and sterile water for injection.

7. Purpose of study

To evaluate the safety and immunogenicity of theSARS-CoV-2 Vaccine (Vero cells), Inactivated in healthy adults aged 18 and above

8. Study design

8.1 Design method

This study is a randomized, double-blind, placebo-controlled clinical trial (phase II)

8.2. Sample size

Following the requirements of "Technical Guidelines for Clinical Trials of Vaccines", there're altogether 1000 subjects taking part in this phase II clinical trial, among which 750 are from the 18-59-year-old group (including 250 in the 0-28-56 d immunization group, 250 in the 0-28 d group, and 250 in the 0-14 d group.) Another 250 subjects are 60 years old and above who take part in the 0-28-56 d immunization group. Sample distribution is shown in Table 1.

Table 1. Grouping and sample distribution of phase II trial

Group	Dosage groups	Number of cases	Vaccine No.	Immunization No.	Immunization schedule		
					First immunization	Second immunization	Third immunization
Aged 18-59 3-dose group	middle-dosage group	100	A2001～A2250	3	0d	28d	56d
	High-dose group	100		3	0d	28d	56d
	Placebo group	50		3	0d	28d	56d
Aged 18-59 2-dose 0-28d group	middle-dosage group	100	A2251～A2500	2	0d	28d	—
	High-dose group	100		2	0d	28d	—
	Placebo group	50		2	0d	28d	—
Aged 18-59 2-dose 0-14d group	middle-dosage group	100	A2501～A2750	2	0d	14d	—
	High-dose group	100		2	0d	14d	—
	Placebo group	50		2	0d	14d	—
Aged ≥60 group	middle-dosage group	100	B2001～B2250	3	0d	28d	56d
	High-dose group	100		3	0d	28d	56d
	Placebo group	50		3	0d	28d	56d
Total		1000					

8.3 Random and blind tests

The clinical study used the test design of random, double-blind, placebo control groups. Randomized statisticians generated random blind bases by using the SAS 9.4 or higher version of software. In each of the immunization groups (no matter from 18-59-year-old group or ≥60-year-old group), the subjects are allocated into medium-dose group, high-dose group and placebo group at a ratio of 2:2:1. After qualified respondents were screened, they were allocated and numbered according to the recruitment order. These respondents were injected with corresponding number of vaccines for study. Experimental vaccine and placebo had the same package and double blinds for the clinical test were realized according to the blind setting method of vaccines.

Randomized statisticians generated standby blind bases of vaccine by using the SAS 9.4 or higher version of software, and prepare backup vaccines according to the proportions of middle dose, high dose and placebo of 2:2:1. When colors of experimantal vaccines are changed or some damages are observed, the vaccination staffs shall report the supervisor and principal investigator to initiate the backup vaccine use program, in which the backup vaccine No. is acquired by the online backup vaccine acquisition system and the backup vaccine is used to replace the research vaccine.

8.4 Vaccine No.

In the clinical test, the applier was responsible for providing qualified experimental vaccines and placebo

and it could authorize the non-blind randomized statisticians to organize vaccine numbering. According to random blind bases, experimental vaccines and placebo were encoded into serial numbers randomly (vaccine per person has the sole serial No.). Moreover, the sole serial No. and other relevant contents shall be marked uniformly on the external package.

Blind bases are kept secretly by two people in duplicates and the coding materials shall be sealed. Blind coding staffs are prohibited to participate in clinical tests and disclose the blind coding contents to people who participate in clinical tests.

Backup vaccine: 100 backup vaccines, including 40 middle-dose vaccines, 8 high-dose vaccines and 20 placebos, were provided except experimental vaccines for injection to the planned respondents. Vaccines which cannot be used shall be replaced by backup vaccines. In the clinical test, when backup vaccine has to be used, the vaccination staffs opened the envelope which agrees with the research No. of the respondents and then injected the backup vaccine to the respondents according to the recorded No. in the envelope.

8.5 Unblinding and emergency unblinding

In this trial, representatives of the sponsors, investigators, and statisticians will unblind the study cohorts together after blinded review of the data at the end of 28 d safety observation and immunogenicity data analysis when the last subjects in different cohorts receive the final dose.

The randomization statistician will prepare contingency letters while blinding, each letter containing a random password for unblinding use. Each random password corresponds to any study number, and the true group of that study number is fed back through the online unblinding system. Each random password represents one chance to unblind only one study number. A random password will expire when used, it is invalid for the unblinded study number. In this study, 10 emergency envelopes are prepared for the phase II clinical trial, which are kept by the person in charge on site. The emergency envelope opening will be checked in the blinded audit.

In case of an emergency (such as a serious adverse event) in the field operation, the investigator may perform an individual emergency unblinding if he or she deems it necessary to clarify information about the experimental vaccine administered to the subject so that appropriate treatment measures can be taken. The person in charge of the site will open and read the emergency letter, log in to the online emergency unblinding system with the unblinding random password inside the letter, and perform emergency unblinding in accordance with the instructions. The investigator involved in the unblinding should sign the name with date and time, and given the reason for unblinding. Investigators should evaluate the relationship between adverse events and the experimental vaccine prior to emergency unblinding. Investigators should immediately notify the sponsor via a 24-hour emergency telephone before conducting emergency unblinding for any reason during the study period. The reason for emergency unblinding must be recorded in the subject's CRF. Subjects unblinded will withdraw from the study, and their safety data will be included in the safety set and possible FAS.

8.6 Experimental vaccine

The experimentalSARS-CoV-2 Vaccine (Vero cells), Inactivated and the placebo were jointly developed by Shenzhen Kangtai Biological Products Co., Ltd. and Beijing Minhai Biotechnology Co., Ltd., and have passed the inspection of the National Institutes for Food and Drug Control.

8.6.1 Experimental vaccines

Medium-dose experimental vaccine: in a liquid dosage form, 0.5 ml/dose, containing 5 μg of SARS-CoV-2 protein.

High-dose experimental vaccine: in a liquid dosage form, 0.5 ml/dose, containing 10 μg of SARS-CoV-2 protein.

8.6.2 Placebo

In a liquid dosage form, 0.5 ml/dose, containing 0.25 mg of aluminum hydroxide adjuvant.

8.7 Clinical endpoints

8.7.1 Primary endpoints

- “the positive seroconversion rate and antibody level of neutralizing antibody against COVID-19 virus 28 days after the whole immunization”
- The positive seroconversion rate of IgG antibody (ELISA method) against COVID-19 virus 28 days after the whole immunization

8.7.2 Secondary endpoints

- Incidence of adverse events within 28 d after each dose of vaccine.

- Incidence of serious adverse events from the first dose to 12 months after full-course immunization.
- The antibody level of IgG antibody (ELISA method) against COVID-19 virus 28 days after the whole immunization
- Positive seroconversion rate and antibody level of neutralizing antibody and IgG antibody (ELISA method) against COVID-19 virus 28 days after the 2nd dose (as for 3-dose immunization group) or 14 days after the whole immunization (as for 2-dose immunization group)
- Positive seroconversion rates and the levels of novel coronavirus neutralizing antibodies and IgG antibodies (ELISA method) at 3, 6 and 12 months after full-course immunization.

8.8 Study plan

8.8.1 Phase II clinical study plan

For each age group, the phase II trial is only permitted, when safety and tolerability have been preliminary confirmed in the phase I trial 7 days after the 1st dose of the high-dose group.

Volunteers will be screened from the resident healthy adults aged 18 years and above in accordance with the principles of voluntary registration, informed consent, and compliance with inclusion and exclusion criteria. When enrolled, every subject will be given a code number and get the correspondingly numbered vaccine shot. Subjects in the 18-59-year-old group will receive the experimental vaccine or placebo according to the 2-dose 0-14 d or 0-28 d immunization schedule, or 3-dose 0-28-56 d immunization schedule, respectively. Subjects in the ≥60-year-old group will receive the experimental vaccine or placebo according to the 3-dose 0-28-56 d immunization schedule. After each dose, the subjects need to stay on site for 30 min to see if there's any immediate response. During the following 7-day systematic follow-up observation, the subjects should record their daily observation results on the Diary Card. After each dose, subjects will receive a telephone visit on day 3 and an intensive visit on day 7 by the researchers, to verify the safety of the vaccination. Subjects should carry out safety observations on their own from days 8-28 after each vaccination. After each dose, subjects will receive a telephone visit on day 14, and an intensive visit on day 28 by the investigator, to verify the safety of the vaccination.

About 5 ml of venous blood will be collected from subjects in the 2-dose immunization group before the 1st dose and 14 and 28 d after whole immunization, and from subjects in the 3-dose immunization group before the 1st and 3rd dose and 28 d after whole immunization, respectively, in order to determine the serum antibody levels. See Tables 2-7 for visit time, time window and visit content for different age groups.

Table 2. Visit content and schedule for 0-28-56 d immunization group

Visit No.		V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13
Content		Pre-immune 0-7 d	Day 0	3-5 d after V1	7-8 d after V1	14-21 after V1	28-35 d after V1	3-5 d after V5	7-8 d after V5	14-21 after V1 天	28-35 d after V5	3-5 d after V9	7-8 d after V9	14-21 after V9	28-35 d after V9
1	Informed consent	X													
2	Collection of demographic information	X													
3	IgG, IgM screening	X													
	RT-PCR assay	X													
	Urine pregnancy test (for women of childbearing age)	X					X				X				
	Height, weight, blood pressure		X												
	Pre-immune body temperature measurement		X				X				X				
4	Vaccination screening		X				X				X				
5	Assignment of study number		X												
6	Blood collection for antibody testing		X								X				X

Visit No.		V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13
Content		Pre-immune 0-7 d	Day 0	3-5 d after V1	7-8 d after V1	14-21 after V1	28-35 d after V1	3-5 d after V5	7-8 d after V5	14-21 after V1 天	28-35 d after V5	3-5 d after V9	7-8 d after V9	14-21 after V9	28-35 d after V9
7	Vaccination		X				X				X				
8	30-min stay for observation, safety observation training		X				X				X				
9	Distribution of safety observation equipment		X				X				X				
10	Safety follow-up visit			X	X	X	X	X	X	X	X	X	X	X	X
11	Review of the Diary Card				X		X		X		X		X		X
12	Collection of the Diary Card						X				X				X
13	Subjects readily report AEs of Grade 3 and above and SAEs		X		X		X		X		X		X		X
14	Record the Original Record Book		X		X		X		X		X		X		X

Table 3. Sample collection schedule for subjects in the 0-28-56 d immunization group

Sample	Visit	V0	V1	V5	V9	V13
	Duration (d)	Pre-immune 0-7 d	Before 1 dose immunization	Before 2 dose immunization	Before 3 dose immunization	28-35 d after 3 dose immunization
Venous blood	Antibody test		5ml		5ml	5ml
Fingertip blood	IgG, IgM screening	Right amount				
Throat swab	RT-PCR assay	Right amount				
Urine sample (for women of childbearing age)	Urine pregnancy test	10ml		10ml	10ml	

Table 4. Visit content and schedule for 0-14 d immunization group

Visit No.		V0	V1	V2	V3	V4	V5	V6	V7	V8
Content		Pre-immune 0-7 d	Day 0	3-5 d after V1	7-8 d after V1	14-21 d after V1	3-5 d after V4	7-8 d after V4	14-21 d after V4	28-35 d after V4
1	Informed consent	X								
2	Collection of demographic information	X								
3	IgG, IgM screening	X								
	RT-PCR assay	X								
	Urine pregnancy test (for women of childbearing age)	X				X				
	Height, weight, blood pressure		X							
	Pre-immune body temperature measurement		X			X				
4	Vaccination screening		X			X				
5	Assignment of study number		X							
6	Blood collection for antibody testing		X						X	X
7	Vaccination		X			X				
8	30-min stay for observation, safety observation training		X			X				
9	Distribution of safety observation equipment		X			X				
10	Safety follow-up visit			X	X	X	X	X	X	X
11	Review of the Diary Card				X	X		X		X

Visit No.		V0	V1	V2	V3	V4	V5	V6	V7	V8
Content		Pre-immune 0-7 d	Day 0	3-5 d after V1	7-8 d after V1	14-21 d after V1	3-5 d after V4	7-8 d after V4	14-21 d after V4	28-35 d after V4
12	Collection of the Diary Card					X				X
13	Subjects readily report AEs and SAEs of grade 3 and above		X		X	X		X	X	X
14	Record the Original Record Book		X		X	X		X	X	X

Table 5. Sample collection schedule for subjects in the 0-14 d immunization group

Sample	Visit	V0	V1	V4	V7	V8
	Duration (d)	Pre-immune 0-7 d	Before 1 dose immunization	Before 2 dose immunization	After 2 dose immunization 14-21 d	After 2 dose immunization 28-35 d
Venous blood	Antibody test		5ml		5ml	5ml
Fingertip blood	IgG, IgM screening	Right amount				
Throat swab	RT-PCR assay	Right amount				
Urine sample (for women of childbearing age)	Urine pregnancy test	10ml		10ml		

Table 6. Visit content and schedule for 0-28 d immunization group

Visit No.		V0	V1	V2	V3	V4	V5	V6	V7	V8	V9
Content		Pre-immune 0-7 d	Day 0	V1 3-5 d	7-8 d after V1	14-21 d after V1	28-35 d after V1	3-5 d after V5	7-8 d after V5	14-21 d after V5	28-35 d after V1
1	Informed consent	X									
2	Collection of demographic information	X									
3	IgG, IgM screening	X									
	RT-PCR assay	X									
	Urine pregnancy test (for women of childbearing age)	X					X				
	Height, weight, blood pressure		X								
	Pre-immune body temperature measurement		X				X				
4	Vaccination screening		X				X				
5	Assignment of study number		X								
6	Blood collection for antibody testing		X							X	X
7	Vaccination		X				X				
8	30-min stay for observation, safety observation training		X				X				
9	Distribution of safety observation equipment		X				X				
10	Safety follow-up visit			X	X	X	X	X	X	X	X
11	Review of the Diary Card				X		X		X		X
12	Collection of the Diary Card						X				X
13	Subjects readily report AEs and SAEs of grade 3 and above		X		X		X		X	X	X
14	Record the Original Record Book		X		X		X		X	X	X

Table 7. Sample collection schedule for subjects in the 0-28 d immunization group

Sample	Visit	V0	V1	V5	V8	V9
	Duration (d)	Pre-immune 0-7 d	Before 1 dose immunization	Before 2 dose immunization	After 2 dose immunization 14-21 d	After 2 dose immunization 28-35 d
Venous blood	Antibody test		5ml		5ml	5ml
Fingertip blood	IgG, IgM screening	Right amount				
Throat swab	RT-PCR assay	Right amount				
Urine sample (for women of childbearing age)	Urine pregnancy test	10ml		10ml		

8.8.2 Long-term Safety and Immune Persistence Study

Subjects will be followed up passively during a 12-month long-term safety observation after whole immunization to verify the incidence of serious adverse events during the study period. A visit (face-to-face/telephone visit) will be conducted once each at 3, 6, 9 and 12 months after full-course immunization to collect the data on the incidence of SAEs.

To observe the immune persistence in subjects who have completed the full-course immunization, about 5 ml of venous blood will be collected at 3 months (+30 d), 6 months (+30 d) and 12 months (+30 d) after the full-course immunization, and the serum will be separated for antibody detection and immune persistence evaluation.

8.9 Study Duration

For each subject it will last about 15 months from enrollment to discharge at the end of the final visit, and some subjects may terminate the study early at some point during the study.

This study will take about 16 months from the time the first subject is enrolled to the time the last subject is discharged at the end of the final visit.

8.10 Study Suspension/Early Termination

Criteria for suspension:

- The trial will be suspended in the event of 1 grade 4 or serious adverse event that may be related to vaccination during the study period;
- The trial will be suspended when more than 15% of subjects have experienced adverse events of grade 3 or above.

If the occurrence of adverse events meets the set criteria for suspension, firstly, the trial shall be suspended. Then, temporary DSMB meeting shall be held,

And the AE shall be reported to the national drug regulatory authority and the ethics committee, and the subject shall be notified. Based on the analysis of the correlation between adverse events, hazard degree and safety risks, investigation reports shall be formed and reported to relevant parties for them to decide whether to restart the clinical trial or not.

Criteria for early termination:

- The trial will be terminated early when there is 1 death that may be related to vaccination during the study period;
- When more than 30% of subjects have adverse events of Grade 3 and above, the investigators, sponsors and the data security monitoring committee will discuss together to decide whether to terminate the trial early;
- The sponsor requests full termination of the trial with justification;
- The administrative authorities request full termination of the trial with justification.
- The ethics committee requests full termination of the trial with justification.

9. Subject Population

9.1 The Selection of Subject Population

The target population is the local health population aged ≥ 18, with the principle of voluntary participation. The volunteers shall participate the study after signing the informed consent form and passing the physical examination and the following inclusion and exclusion criteria screening. Investigators implementing the study, relevant study personnel and any employees of the contract research organizations (CROs) shall not be the subjects.

9.2 Inclusion Criteria

- Resident healthy adults aged 18 years and above;
- Volunteers give their consent and sign an informed consent form;
- Volunteers comply with the requirements of the clinical trial protocol.
- Axillary temperature ≤ 37.0℃.
- Female subjects of childbearing potential are not pregnant or breastfeeding at enrollment, and have no plans to have a baby within the first 3 months of enrollment; and they take effective contraceptive measures within 2 weeks prior to enrollment.

9.3 Exclusion Criteria

- Those subjects who have traveled abroad or have been to a village/community where an outbreak has occurred within 14 days prior to vaccination, have been exposed to COVID-19 infected or suspected cases, or are in quarantine, or come from their village/community where there are COVID-19 infected or suspected cases;
- Confirmed or suspected cases, or asymptomatic infections of COVID-19 (check the China Disease Prevention and Control Information System);
- Self-reported history of SARS virus infection;
- Positive RT-PCR test results on pharyngeal swabs;
- Those with a self-reported history of SARS virus infection;
- Those with previous severe allergic reactions to vaccination (e.g., acute allergic reactions, urticaria, skin eczema, dyspnea, angioneurotic edema, or abdominal pain) or allergic to known components of the inactivated novel coronavirus pneumonia (COVID-19) vaccine;
- Those with a history or family history of convulsions, epilepsy, encephalopathy or mental illness;
- Those with congenital malformations or developmental disorders, genetic defects, or severe malnutrition, etc.;
- Those known or suspected to have diseases including: severe respiratory disease, severe cardiovascular disease, severe liver or kidney disease, medically uncontrollable hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg; systolic blood pressure ≥ 150 mmHg and diastolic blood pressure ≥ 100 mmHg for subjects aged ≥ 60 years), complications of diabetes mellitus, malignancy, various acute diseases or acute episodes of chronic disease;
- Those diagnosed with congenital or acquired immunodeficiency, HIV infection, lymphoma, leukemia or other autoimmune diseases;
- Those with a history of coagulation abnormalities (such as deficiency of coagulation factors, coagulopathies);
- Those on anti-tuberculosis treatment;
- Those who have received other investigational drugs within 6 months prior to vaccination;
- Those who have received immune enhancement or inhibitor therapy within 3 months before vaccination (continuous oral or instillation for more than 14 d);
- Those who have received blood products within 3 months before vaccination;
- Those who have received a live attenuated vaccine within 14 days before vaccination;
- Those who have received other vaccines within 7 d before vaccination;

- Those with other conditions not eligible for participating in the study at the investigator's discretion.

9.4 Exclusion Criteria for Subsequent Vaccinations

After the enrollment, during the study period, if the COVID-19 epidemiological history, suspected COVID-19 clinical manifestations appear, epidemiological investigation and nucleic acid tests shall be carried out immediately, chest CT shall be taken if necessary, and the subjects shall undergo quarantine observation according to local epidemic prevention and control. If the subject is diagnosed with a COVID-19, he/she shall withdraw from the clinical trial, and accept the reasonable treatment from local center for disease control as required.

If one of the following (1) to (3) adverse events (AE) occurs, the vaccination shall be prohibited, other research procedures can be continued according to researchers' discretion; If one of the following (4) to (5) adverse events occurs, it is up to the investigator to determine whether the vaccination shall be continued; If one of the following (6) to (7) adverse events occurs, the vaccination shall be delayed within the time window specified in the protocol.

(1) Any serious adverse events with cause association with vaccination;
(2) Serious allergic response or hypersansitivity after the vaccination (including urticaria/rash within 30 minutes after vaccination);
(3) Any diagnosed or suspected auto-immune diseases or immunodeficiency diseases (including human immunodeficiency virus (HIV) infection).
(4) Emerging chronic diseases after vaccination or acute attack of chronic diseases;
(5) Severe intolerable adverse reaction occurs.
(6) Suffering from acute diseases at vaccination (acute diseases refer to moderate or severe diseases with or without fever);
(7) Axillary temperature ≥37.0 ℃ at vaccination.

9.5 Withdrawal or Discontinuation Criteria

If the subjects prematurely terminate the research, the researcher should record and dispose as follows:
(1) Loss contact.
(2) Death, the investigator shall record the reasons for death.
(3) Voluntarily withdrawal, the investigator shall record the situation and the reasons for withdrawal:

- Subjects advocated prematurely study termination (including vaccination termination, collection of biological specimen and safety observation, etc.), research data before withdrawal can be used for analysis. If the subject prohibits the investigator to continue to use all relevant research data, research data before withdrawal cannot be used for analysis.
- The subjects advocate early termination of part of the study, for example, only stop the vaccination, or only stop the collection of biological specimens, other studies specified in the protocol shall be completed.
- Subjects withdraw for reasons related to research, the specific withdrawal reason shall be recorded, researchers shall follow up the subjects who withdraw due to AE / SAE until the event was resolved.
- Subjects withdraw for reasons unrelated to research (including out for a long time, migration), The investigator shall record the reasons for withdrawal.

(4) Investigator exclusion

Subjects meet the exclusion criteria, emergency unblinding criteria, or investigator consider not suitable for participating in the research, the reason for exclusion shall be recorded.

10. Methods and Procedures

10.1 The Selection of Subjects

The local healthy volunteers aged ≥ 18 shall be enrolled, and provided with oral and written information of the study, and asked to sign the informed consent form.

After the signing of informed consent, the volunteers were screened according to the inclusion and exclusion criteria, qualified subjects can be registered into the group and assigned research number. Subjects from different age groups will be assigned to the corrsponding study number according to the age from young to old.

10.2 Clinical Study Grouping and Number of Population Inoculated

See “8.2 Sample Size”.

10.3 Inoculation

10.3.1 Storage and Transportation of the Vaccines

The vaccines shall be stored and transported at 2 ~ 8℃, protected from freezing.

10.3.2 Inoculation Methods and Precautions

Observation objects that meet the inclusion criteria can be vaccinated, information of the experimental vaccine and the information of the subjects should be checked before vaccination, vaccines meet the requirements of the clinical trial protocol can be inoculated.

Inoculation site and route: inoculation site is lateral triangular muscle of upper arm, inoculation route is intramuscular injection.

The injection site shall be disinfected with 75% alcohol before injection, experimentalexperimental vaccines shall be injected intramuscularly after the skin is slightly dry. The vaccine shall be well shaken before use. Experimental vaccines shall not be injected intradermally or subcutaneously. Subjects shall be carefully observed for at least half an hour after vaccination with the experimental vaccine, and appropriate emergency medical treatment measures should be prepared fir serious allergic response after the vaccination.

10.3.3 Concomitant Medications and Concomitant Vaccination

Allowed medications: if the subjects experience AE during the clinical trial, necessary drug treatment is allowed, the name, administration route, reason and medication time shall be recorded.

Allowed vaccination: during the study period, subjects can receive routine immunization according to the product instruction, however, there needs an interval between routine inoculation and the previous dose of the experimental vaccine, it is 7 days for subunit vaccine and non-live vaccine, 14 days for live attenuated vaccine. Emergency vaccination for medical treatment is allowed, for example, rabies or tetanus vaccines can be timely inoculated according to the instructions. The name, usage, time and the vaccination site of concomitant vaccination shall be recorded.

10.4 SafetyObservation

10.4.1 Definition of Adverse Events and Adverse Reactions

Adverse events: refer to all adverse medical events occurring to the subjects after receiving the investigatinal drugs, which are shown in symptoms, diseasesor laboratory abnormalities. However, it is not always possible to deduce causal relationship between them and the experimental vaccines.

Adverse reactions: refer to all hazard or unexpected reactions for human that may be related to the experimental vaccines in clinical trials. There is at least a reasonable probability of a cause relationship between the experimental vaccines and the adverse event, that is, the correlation cannot be ruled out.

Serious adverse event: refer to all adverse medical events occurring to the subjects after receiving the investigatinal drugs, which are shown in death, life-threatening, permanent or serious disability, or the subjects need hospitalization or extended hospitalization and congenital abnormality or birth defect.

10.4.2 Oberservation time and method of adverse reaction

Subjects should be observed for immediate response at the inoculation site for 30 min after each must dose of vaccine, local / systemic adverse events within this period shall be recorded. Emergency equipment, drug and healthcare professional shall be available on site. The investigator shall closely follow the subjects within 7 days after each vaccination, ask the subjects to carry out safety observations on their own, the observation results shall be recorded on the daily diary card by the subjects. Subjects will be visited intensively by the investigator at day 7 after each vaccination to verify safety observations. Subjects shall carry out safety observations on their own from days 8-28 after each vaccination, and record the observation results on the daily diary card. Subjects will be visited intensively by the investigator at day 28 after each vaccination to verify safety observations.

During the study period, the serious adverse events shall be observed by initiative report of the subjects and regular visits of the investigators. The researchers shall carry out investigation and verification, and handle well after receiving serious adverse events reports A visit (face to face/telephone visit) will be conducted once each at 3, 6, 9 and 12 months after immunization to collect the data on the incidence of SAEs.

10.4.3 Safety observation indexes

Local reaction: pain, induration, swelling, rash, dizziness, pruritis, cellulitis.

Systemic reactions (including vital sign): fever, diarrhea, anorexia, vomiting, nausea, muscle pain, headache, cough, dyspnea, abnormal skin mucosa, acute allergic reaction, fatigue.

10.4.4 Safety assessment criteria

Adverse events after vaccination shall be judged according to grading criteria of *Guiding Principles of Grading Standards for Adverse Events in Clinical Trials of Preventive Vaccines* (National Drug Administration No. 102 of 2019)

Table 8. Rating Scale of Reaction Events at the Inoculation Site (Local)

Symptom/sign	Grade 1	Grade 2	Grade 3	Grade 4
Pain	No or minor impact on physical activities	Impact on physical activities	Impact on daily life	Loss of basic self-care ability, or hospitalization
Induration*, swelling**#				
Aged >14	2.5-＜5 cm in diameter, or 6.25-＜25 cm^2 in area, and not or minor impact on daily life	5-＜10 cm in diameter or 25-＜100 cm^2 in area, and impact on daily life	≥10 cm in diameter, ≥100 cm^2 in area, ulceration, secondary infection, phlebitis, aseptic abscess, wound drainage, or severe impact on daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Rash*, areola**#				
Aged >14	2.5-＜5 cm in diameter, or 6.25-＜25 cm^2 in area, and not or minor impact on daily life	5-＜10 cm in diameter or 25-＜100 cm^2 in area, and impact on daily life	≥10 cm in diameter, ≥100 cm^2 in area, ulceration, secondary infection, phlebitis, aseptic abscess, wound drainage, or severe impact on daily life	Abscess, exfoliative dermatitis, dermal or deep tissue necrosis
Pruritus	Pruritus at the inoculation site, relieved spontaneously or within 48 hours after treatment	Pruritus at the inoculation site, not relieved within 48 hours after treatment	Impact on daily life	NA
Cellulitis	NA	Non-injection therapy (e.g., oral antibacterial, antifungal and antiviral medication) is required	Intravenous injection therapy (e.g., intravenously antibacterial, antifungal and antiviral medication) is required	Sepsis, or tissue necrosis, etc.

Note: *In addition to measuring the diameter directly for grading evaluation, subsequent changes to the measurement results should also be recorded.

**The maximum measured diameter or area should be used.
#The indicators with higher grades should be selected based on the functional grade and actual measurement results for the evaluation and grading of induration, swelling, rash and areola.

Table 9. Vital Sign Rating Scale

Vital signs	Grade 1	Grade 2	Grade 3	Grade 4
Fever* [axillary temperature (℃)]				
Aged >14	37.3～＜38.0	38.0～＜38.5	38.5～＜39.5	≥39.5, lasting for more than 3 days

Note: *Axillary temperature is usually adopted in China, and converted to oral and rectal temperature when necessary. Generally, oral temperature = axillary temperature +0.2℃; rectal temperature = axillary temperature + (0.3-0.5℃). The cause should be identified early in case of a persistent high fever.

Table 10. Rating Scale of Adverse Events at the Non-inoculation Site (Systemic)

Symptom/sign of organ system	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal system				
Diarrhea	Minor or transient, 3-4 times/day, abnormal stool appearance, or minor diarrhea for less than 1 week	Moderate or persistent, 5-7 times/day, abnormal stool appearance, or diarrhea for ＞ 1 week	＞7 times/day, abnormal stool appearance, or hemorrhagic diarrhea, orthostatic hypotension, and electrolyte imbalance, which require intravenous infusion＞2L	Hypotensive shock, which requires hospitalization
Anorexia	Loss of appetite, but without decrease of food intake	Loss of appetite, with decrease of food intake but no significant weight loss	Loss of appetite and significant weight loss	Interventions are required (e.g., gastric tube feeding and parenteral nutrition)
Emesis	1-2 times/24 hours and no impact on activities	3-5 times/24 hours or imitation of activities	＞6 times within 24 hours or need for intravenous rehydration	Need for hospitalization or other means of nutrition due to hypotensive shock
Nausea	Transient (<24 hours) or intermittent, and generally normal food intake	Persistent nausea results in decreased food intake (24-48 hours)	Persistent nausea results in almost no food intake (>48 hours) or the need for intravenous rehydration	Life threatening (e.g., hypotensive shock)
Musculoskeletal and connective tissue				
Myalgia (non-inoculation site)	No impact on daily activities	Slight impact on daily activities	Severe myalgia, with severe impact on daily activities	Emergency or hospitalization
Nervous system				
Headache	No impact on daily activities and no need for treatment	Transient; slight impact on daily activities and possible need for treatment or intervention	Severe impact on daily activities and need for treatment or intervention	Refractory; need for emergency treatment or hospitalization
Respiratory system				
Cough	Transient; no need for treatment	Persistent cough, with effective treatment	Paroxysmal cough, with uncontrollable treatment	Emergency or hospitalization
Dyspnea	Dyspnea during exercise	Dyspnea during normal activities	Dyspnea at rest	Dyspnea, and need for oxygen therapy, hospitalization or assisted breathing
Skin and subcutaneous tissue				
Abnormal skin and mucous membranes	Erythema/pruritus/color changes	Diffuse rash/maculopapule/dryness/desquamation	Blister/exudation/desquamation/ulcer	Exfoliative dermatitis with mucosal involvement, erythema multiforme, or suspected Stevens-Johnsons syndrome
Immune system				
Acute anaphylases**	Local urticaria (blisters) not requiring treatment	Local urticaria requiring treatment, or minor angioedema not requiring treatment	Extensive urticaria or angioedema requiring treatment, or minor bronchospasm	Anaphylactic shock, life-threatening bronchospasm or laryngeal edema
Others				

Fatigue	No impact on daily activities	Impact on normal daily activities	Severe impact on daily activities and inability to work	Emergency or hospitalization

Note: ** refers to type I hypersensitivity.

For adverse events not covered in the above scale, the intensity assessment should be performed according to the following criteria:
Grade 1 Mild: transient (<48h) or slight discomfort, which does not affect activities and not require treatment;
Grade 2 Moderate: mild or moderate activity limitation, which may require medical attention, no treatment, or only mild treatment;
Grade 3 Severe: significant restriction of activities, which requires medical attention, treatment, and possibly hospitalization;
Grade 4 Critical: it may be life-threatening, severely restricts activities, and requires monitoring and treatment.
Grade 5 Death

10.4.5 Outcomes of adverse events

The outcomes of adverse events include: (1) recovery, (2) non-recovery, (3) recovery with sequelae, (4) death, (5) loss of follow-up/unknown.

10.4.6 Relationship between adverse events and experimental vaccines

Certainly unrelated: other factors contribute to the occurrence of an adverse event, and there is sufficient evidence that the adverse event/event is caused by other factors instead of vaccination.
Possibly unrelated: an adverse event may be caused by other factors, such as the subject's clinical condition, other treatments or concomitant medications, and it is inconsistent with known adverse reactions of vaccination.
Possibly related: an adverse event is consistent with known information of the experimental vaccine, follows a reasonable time sequence with vaccination, and/or has occurred previously during vaccination. Moreover, it has a causal link with the experimental vaccine, but may also be related to other factors.
Very likely related: an adverse event is consistent with known information of the experimental vaccine, and has a causal relationship with the experimental vaccine which cannot be explained by other factors, such as the subject's clinical conditions, other treatments, or concomitant medications.
Certainly related: an adverse events is consistent with known information of the experimental vaccine, and has a causal relationship with the experimental vaccine which cannot be explained by other factors, such as the subject's clinical conditions, other treatments, or concomitant medications. In addition, adverse events are repeated when subjects are administered with the experimental vaccine again.
Adverse events that are certainly unrelated and possibly unrelated are counted as unrelated to the inoculation of experimental vaccine; while adverse events that are possibly related, very likely related and certainly related are counted as related to the inoculation of experimental vaccine.

10.4.7 Report of severe adverse events

Upon becoming aware of any severe adverse events (SAE), the investigator should promptly (within 24 hours) report all SAEs in writing to the sponsor and submit follow-up reports.
Upon receipt of safety-related information from any source, the sponsor should conduct an analytical assessment, including severity, association with the experimental drug, and identification of an expected event. The sponsor should report suspicious and unexpected severe adverse reactions (SUSAR) to the investigator, the clinical trial institution and the ethics committee as soon as possible. Meanwhile, the sponsor should report SUSARs to the National Medical Products Administration and the health authorities.For fatal or life-threatening SUSARs, the sponsor should report to the National Medical Products Administration immediately after first being informed and within 7 natural days, and report relevant follow-up information within the following 8 days; for non-fatal or non-life-threatening SUSARs, or information about other potential severe safety risks, the sponsor should report to the National Medical Products Administration immediately after first being informed and within 15 natural days.

10.4.8 Medical management of adverse events

Subjects should be informed to record clinically significant adverse events at any time. Early suspected symptoms of COVID-19 (such as fever, dry cough, fatigue, nasal congestion, rhinorrhoea, pharyngalgia, myalgia, diarrhea, polypnea, and dyspnea), COVID-19 infection, grade 3 or above AEs and SAEs, if any, should be reported, at all times, to the investigator who should conduct investigation, verification and follow-up after being informed, until such events are resolved, and finally complete the detailed case investigation and follow-up records. In case of severe or fatal COVID-19 infection, further thematic investigations are required to determine the presence of ADE/VED. If subjects experience grade 3 or above AEs or SAEs after vaccination, the investigator should provide them with appropriate treatment or medical consultation in a timely manner to alleviate or relieve their suffering. If necessary, the green channel for medical treatment should be initiated to ensure timely medical treatment, and the medical measures and results should be recorded in detail during this process.

Adult female subjects are required to take effective contraceptive measures within 2 weeks before enrollment and 1 month after full-course vaccination. If an unplanned pregnancy occurs in subjects between enrollment and 1 month after full-course vaccination, the investigator should immediately stop vaccination, prematurely terminate all studies, and record it as an AE. The investigator should follow up the health of both subjects and children up to 12 months after birth.

10.5 Immunogenicity and cellular immunity evaluation

10.5.1 Blood sample collection

Blood sample collection schedule, see “8.8 Study Plan”. About 5 ml of venous blood need to be collected from subjects to test the immunogenicity.

10.5.2 Blood sample processing and storage

The venous blood collected for immunogenicity testing needs to be centrifuged and separated in time on the same day. The serum should be divided into 4 tubes with at least 0.5ml in each tube and frozen at -20℃ for unified examination. Sera in tube 1 (set A) and tube 2 (set B) are used for antibody testing. Sera in tube 3 (set C) and tube 4 (set D) are available as reserve samples, which will first be kept by the testing team on site, until the immunogenicity test report is issued and verified to be correct. Biological samples collected in this study will be properly stored as required and will be used only for the research tests specified in the protocol. After the use of biological samples, except the remaining serum, the remaining samples will be destroyed by the testing unit in accordance with the relevant provisions. If any additional testing of the remaining serum is required, the approval of the ethics review committee and the subjects’ consent must be sought.

10.5.3 Antibody detection

The neutralization antibody should be detected by microcytopathic method. The China National Institutes for Food and Drug Control is entrusted to detect neutralizing antibody concentrations of pseudovirus, and the Chinese Center for Disease Control and Prevention to detect neutralizing antibody concentrations of live viruses. The China National Institute for Food and Drug Control is entrusted to detect IgG antibody by ELISA.

10.5.4 Evaluation indexes and criteria

The positive seroconversion rate of SARS-CoV-2 antibody, GMT (or GMC), and GMI are taken as the evaluation indexes of immunogenicity.

Determination criteria of positive seroconversion of neutralizing antibodies: negative if antibody titer < 1:4; positive if antibody titer ≥ 1:4. The antibody positive seroconversion is considered if antibody titer is ＜1∶4 before immunization and ≥ 1∶4 after immunization, or antibody titer is ≥ 1:4 before immunization and the antibodies increase by ≥ 4 fold increase after immunization.

10.6 Data management

10.6.1 Data collection, recording and reporting

All test data should be recorded in the electronic data collection (EDC) system and made available to the sponsor. Only authorized investigators and researchers are allowed to access the system during the experiment. All data in the EDC system comes from source files. All modifications to source files are underlined above the original section with a signature and date next to the modified content. Any modifications to data in the EDC system will be recorded by the system. Source files include informed consent, original record books, diary cards, contact cards, etc., which should be properly kept by the investigator.

10.6.2 Monitoring of data records

The supervisor should monitor the data records periodically and irregularly until all data entry, verification and cleaning are completed. Data verification by the supervisor mainly refers to consistency verification, namely, to check the consistency between the data in the EDC system and the original records. The data administrator performs logical checks on the data.

10.6.3 Data quality control

A final data verification should be performed after all data entry, verification and cleaning are completed and before the database is locked.

10.6.4 Database lock

Blind verification is required before statistical analysis. Blind verification mainly determines the population to be analyzed according to evaluation criteria, including FAS data set, PPS data set and safety analysis data set under the ITT principle, and determines the violation/deviation of the scheme and its impact on the analysis data set. Blind verification should be performed by the data administrators with the participation of the investigator and the program leader or their representatives. In addition to the above data review, the main investigators, statistical analysts, data administrators and the sponsor should further discuss and confirm the statistical analysis plan based on the main contents of the experimental scheme. After blind verification is confirmed, the database will be locked, and the statistical analysis plan will be determined simultaneously.

10.7 Statistical considerations

10.7.1Statistical analysis plan

The statisticians are entrusted by the sponsor to undertake the task of statistical analysis and participate in the whole process from experimental design, implementation to analysis and summary. After the experimental scheme is formulated and approved by the ethics committee, the statisticians will write and formulate a statistical analysis plan to define the details of statistical analysis.

10.7.2 Sample size considerations

In the phase II trial:

Assume that the positive seroconversion rate of the vaccine group is 80%, that of the placebo group is 30%, and the ratio between vaccine and placebo is 2:1, while the test level is set to bilateral 0.05, if there’re 100 subjects enrolled in the vaccine group and 50 subjects enrolled in the placebo group, the difference between both groups can be determined with 99.99% certainty. Therefore, for each immunization schedule of each age group in the phase II trial, 100 subjects are enrolled in the mid-dose group, 100 in the high-dose group and 50 in the placebo group respectively.

10.7.3 Analysis data set

10.7.3.1 Safety set

Safety set (SS): all subjects who have received at least one dose of the experimental vaccine are included. For those with wrong vaccination number, safety evaluation will be conducted according to the vaccine group actually administered by the subjects following the principle of ASaT (All Subjects as Treated). SS is mainly used for safety evaluation of vaccines.

This experiment will define the SS of each dose separately based on the safety analysis after different doses of vaccination.

10.7.3.2 Immunogenicity evaluation data set

Full analysis set (FAS): all subjects who follow the principle of intention analysis (ITT), have been randomized, have completed at least one dose of vaccination, completed the pre-immunity evaluation of blood sampling, and have valid antibody values are included. The subjects with incorrect vaccine number should be evaluated for immunogenicity according to their primary randomized group.

Per protocol set (PPS): all subjects who have not violated the inclusion/exclusion criteria, have been randomized, have completed the full-course vaccination, completed all the blood collection before and post vaccination for immunogenicity evaluation, and valid antibody values are included. Among them, subjects meeting the following conditions are not allowed to enter PPS:

1）Those with severe information and data missing after randomization;

2）Those who have seriously violated the protocol;

3）Those who have received the wrong inoculation or incorrect dose;

4）Those who have received concomitant medications or concomitant vaccines that affect the immunogenicity evaluation.

The corresponding PPS should be defined for each time point of immunogenicity evaluation in the basic immunization stage. FAS and PPS are mainly used to evaluate the immunogenicity of vaccines.

Immune persistence set (IPS): all subjects who have undergone the immune persistence evaluation stage and completed blood sampling at the time point for immune persistence evaluation and have antibody values are included. The immune persistence set should be defined for 3 months, 6 months, and 12 months after vaccination.

10.7.4 Statistical analysis method

10.7.4.1 General principles

Measurement data are statistically described by the mean, median, standard deviation, maximum value and minimum value; counting data or grade data are described using frequency number and frequency. All statistical analyses are done with statistical software SAS 9.4 or higher version.

10.7.4.2 Subject disposition and demographic information

The number of subjects enrolled in each group, the number of subjects who completed the trial and the number of cases in each analysis set are summarized respectively and the subjects who dropped out and the reasons for dropout are analyzed. The demographic characteristics of subjects in each group are statistically described.

10.7.4.3 Immunogenicity assessment

The positive rate and positive seroconversion rate of the COVID-19 antibody after immunization are calculated respectively, the bilateral 95% confidence interval is calculated by Clopper-Pearson and the differences between groups are statistically tested by chi-square test/Fisher's exact test.

The differences between groups in the COVID-19 antibody GMT of subjects after immunization, the antibody after immunization and its growth compared with that before immunization are statistically tested by analysis of variance after logarithmic transformation using the geometric mean and 95% confidence interval.

10.7.4.4 Safety evaluation

AE is medically coded by MedDRA and counted according to the classification of SOC and PT. In addition, the collective adverse events will be counted according to the classification of systemic reaction and local reaction as specified by the protocol. The treatment emergency adverse events (TEAE) after inoculation are mainly counted and analyzed in this trial, that is, the AE occurred after first dose inoculation or the AE with aggravated severity after first dose inoculation. The AE incurred before first dose inoculation is listed. Unless otherwise stated, the following AE is TEAE.

The occurrence number, number of cases and incidence rate of all AE in each group, AE related to the vaccine study, AE unrelated to the vaccine study, AE of Grade 3 or above, AE of Grade 3 or above related to the vaccine study are calculated. The dose distribution, time distribution and severity of AE are statistically described. The list of AE related to the vaccine study, list of AE unrelated to the vaccine study and list of AE of Grade 3 and above are set out.

The occurrence number, number of cases and incidence rate of all AE in each group, AE related to the vaccine study and AE unrelated to the vaccine study are calculated, and the differences between groups are statistically compared by Fisher's exact test. The SAE list is set out.

10.7.5 Interim analysis

At present there's no plan to conduct a mid-term safety analysis. If such an analysis does take place later, independent statisticians from DSMB will issue an unblinded safety report accordingly. The safety evaluation will be carried out by DSMB. The interim analysis in this study is for safety analysis only and does not affect class I errors in the trial.

10.7.6 Processing of missing data

The missing data after inoculation of the immunogenicity endpoint neutralization antibody results are imputed by LOCF in this trial and the indexes such as antibody positive rate and positive seroconversion rate are further calculated derivatively. Processing of missing data in the safety endpoint is not considered. See the statistical analysis plan for the details of processing of missing data.

10.8 Initial analysis and additional analysis

Initial analysis: After the last subject completes the visit and immunogenicity test on Day 28 after the last immunization, all parties jointly complete the data review and database locking before unblinding for statistical analysis.

Updated analysis: Long-term safety observation and statistical analysis of immune persistence will be performed after completion of SAE observation at 12 months after full-course immunization and completion of antibody detection for immune persistence observation at 3, 6 and 12 months after full-course immunization.

10.9 Data safety monitoring board

A Data Safety Monitoring Board (DSMB) will be established in this study to supervise the study safety and enforcement in order to protect the ethical and safety interests of the subjects and the scientificity of the study. The DSMB is composed of clinical experts, vaccine epidemiologists and statistical experts independent of the sponsor, and none of them has any significant conflict of interest with the sponsor. The DSMB will assess the safety data of the trial (for the safety assessment time point in the interim analysis section) to ensure the risk/benefit ratio acceptable to the subjects in the treatment group. The DSMB will advise the sponsor on the measures it deems necessary to protect the subjects and on the overall safety assessment. The sponsor will make an analysis and make a decision on the continuation, revision or termination of the study according to the advice of the DSMB.

The meeting arrangements, composition, responsibilities and management of the DSMB will be described in a separate DSMB charter.

11. Monitoring of clinical trial

11.1 Responsibilities of all parties

The sponsor shall implement and maintain the quality assurance and quality control system and write the quality management documents to ensure that the trial is carried out in accordance with the regulations. The data, records and reports shall meet the requirements of the GCP and other regulations and protocols. Prior to the on-site registration of subjects, the investigator and the CRA will review the clinical protocol and all trial procedures, including information on the vaccine for the trial, procedures for obtaining informed consent, procedures for reporting adverse reactions/events, and procedures for completing the CRF and data management.

The principal investigator shall manage and clearly assign responsibilities to all participants in the clinical trial, and formulate the SOP for each group of the trial.
The personal information of the subjects shall be kept confidential. The CRF form or other documents provided to the sponsor shall be identified only by the subject code and random number. The identification lists and screening registration forms of the subjects (including complete name, age, and address) will be kept in the research files by the researchers. In accordance with GCP principles, the original data of each subject shall be allowed to be monitored, audited and reviewed.
The CRA shall conduct the on-site follow-up monitoring at a regular time interval, check the consistency of the original medical records with the information on CRF, their accuracy and completion in the monitoring and, in case of inconsistency or failure to complete CRF in time, urge the investigator to modify or complete as soon as possible. The CRA will evaluate the process of informed consent, the transportation and storage of vaccine for the trial, the documents for the trial and the progress of the trial
The inspector should check the researchers' compliance with the protocol (or protocol amendment), observe the trial procedure and discuss with the researchers when certain problems occur. make the monitoring logs to record the on-site monitoring and, after the trial, provide the sponsor with a copy of the monitoring record.

11.2 Personnel training

Prior to the start of the study, the sponsor and the principal investigator shall train the participants in the trial by meeting in terms of the summary protocol of the clinical trial, the trial implementation procedures, time arrangement, operation cautions and trial data filling. Any additional CRA or investigator in the trial process shall be trained separately. If the sponsor or the principal investigator deems it necessary, retraining can be conducted. Each training shall be recorded.

11.3 Calibration of instruments and equipment and standardization of thermometer and glassware

- The refrigerator is provided with a temperature record;
- The themometer has been standardized;
- Syringes used for vaccine injection and blood collection are disposable sterile syringes. The manufacturer has the national production license, and the batch number and expiry date are recorded.

11.4 Vaccine management

A special person shall be assigned to manage the vaccine for the trial, establish the vaccine management system and record system and accept the supervision of the CRA in terms of vaccine handover, warehousing and storage, distribution and recovery records.
The sponsor shall provide an adequate quantity of vaccines, including backup vaccinebackup vaccines. The vaccine packaging must meet the requirements of the clinical trial, especially the requirements of the blind design of this study. The vaccine with damaged inner package or deteriorated vaccine shall not be used and shall be returned to the sponsor. If the cold chain system is damaged or the vaccine is frozen in the process of transportation and storage, the vaccine cannot be used. It shall be stored separately and marked with "X", managed by a special person and returned to the sponsor.
At the end of the trial, the investigator shall count all remaining vaccines. The total number of remaining vaccines and vaccines used shall be the same as the number of vaccine received by the investigator, and the remaining vaccine and their outer packages shall be returned to the sponsor.

11.5 Quality control of biological samples

The collected blood samples shall be subject to separation of serum under aseptic conditions, with the hemolysis rate ≤2%, contamination rate ≤1% and error rate ≤1%.

After separation of serum, the serum shall be cryopreserved below -20℃ and the temperature shall be below 0℃ during transportation of the serum samples.

11.6 Quality control of documents

11.6.1 Original Materials

The original data shall include the demographic information, medical history inquiry results, physical examination results, laboratory examination results, vaccination record, blood collection records, concomitant drug, adverse events/reactions of the subjects and their processing and outcome. All information shall be recorded in the original logbook, which shall be properly kept by the investigator in a special room. The original data shall be archived in the research center, which is the basis for the subjects' participation in the clinical trial and the authenticity and integrity of the data.

The investigator shall fill in the original logbook carefully, accurately and timely, and all original data collected shall be recorded in the original logbook with a black sign pen on the same day. Mistakes shall be crossed out instead of be altered directly. The correct content shall be filed in next to it, signed and dated.

11.6.2 Sample case report form (CRF)

The electronic CRF is established by the "online electronic medical record entry system (EDC)" in this trial. Only the investigator and authorized staff will be allowed to access the EDC system during the trial. The electronic CRF is used to record the data of the clinical trial and is an important part of the clinical trial and study report. It shall be filled in with normative language according to the EDC system instructions and CRF filling instructions. Any electronic CRF data entry, verification, modification, cleaning and quality control process will be recorded in the EDC system. After data cleaning, the investigator shall confirm and electronically sign the data in each CRF.

All data on the electronic CRF are derived from and consistent with the original materials. All data recorded in the CRF shall be recorded in the original materials.

The CRA authorized by the sponsor shall have access to the CRF, informed consent form and all original data at any time.

Any amendments to the clinical trial exchanges, meetings, protocols and SOP by the sponsor, investigator and other relevant personnel shall be provided in writing. All documents agreed upon by both parties shall be made out in duplicate, saved and filed respectively.

11.6.3 Data retention

All data in the test trial shall be retained by the investigator for at least five years after the termination of this clinical trial according to the *Guideline for Retention of Essential Documents for Drug Clinical Trials*. The sponsor should retain clinical study data for at least five years after the approval for marketing of the experimental drug.

12. Ethical approval

12.1 Ethical review and approval

The clinical trial shall be approved by the local ethics committee. The principal investigator shall submit the clinical trial protocol and all necessary additional documents and relevant information to the ethics committee. Upon approval of the ethics committee, the investigator shall provide the sponsor with a copy of the ethics committee approval.

12.2 Supervision by IEC

Throughout the trial, the IEC shall supervise whether there are ethical issues that harm the subjects and whether the subjects are treated or compensated for the harm caused by the trial, and evaluate the extent of risk to which the subjects are exposed.

13. Confidentiality

The sponsor, investigator, IEC or the representative of a fully authorized regulatory body, such as CFDA, shall have the access to the data relating to the clinical trial, provided that such information shall not be used in any other clinical trial, nor shall it be disclosed to any other individual or entity.

The investigator must sign a confidentiality agreement to confirm that he/she knows and agrees that he/she is responsible for the confidentiality of the information in the study. The investigator and other researchers shall keep confidential all information provided by the sponsor and all data/information generated at the research center (other than the medical records of the subjects). Such information and data shall not be used for any purpose other than study. This limitation does not apply when (1) the study information is not made public for the violation by the investigator and researchers; (2) the study information is only disclosed to the IRB/IEC for the purpose of study evaluation; (3) the study information is made public in order to provide appropriate medical assistance for subjects; (4) the study results authorized by the sponsor are published. If the confidentiality clause involved in the written contract for this study conflicts with this statement, the terms of the contract shall prevail.

14. Potential hazards and hazard minimization

14.1 Vaccination

Subjects are not required to pay any fees for participating in this clinical trial, including physical examination, vaccination, and scheduled outpatient visits. During the study period, subjects will be compensated for transportation, lost labor, nutrition, etc. Subjects may be protected by the vaccine against COVID-19 for a period of time. Vaccination may cause some common adverse reactions. According to the literature, the incidence of adverse reactions to similar vaccines is very low and the reactions are slight. These adverse reactions may include pain, redness, swelling, fever, headache, nausea, vomiting, fatigue and other symptoms at the vaccination site. The subjects may get timely medical treatment in case of any serious adverse event related to or possibly related to the vaccination and when necessary, the “green channel for medical treatment” is started for emergency treatment.

14.2 Sample collection

After qualification, the experienced medical staff shall trained to collect venous blood according to the prescribed procedures to minimize pain or danger (including pain and rare venipuncture site infection) to subjects.

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