A CLINICAL TRIAL OF HETEROLOGOUS BOOSTER IMMUNIZATION WITH RECOMBINANT COVID-19 VACCINE (ADENOVIRUS TYPE 5 VECTOR) FOR INHALATION AFTER PRIMARY SERIES OF INACTIVATED COVID-19 VACCINE

Project No.: Protocol No.: JSVCT137

Study Centers: Jiangsu Provincial Center for Disease Control and Prevention (Public Health Research Institute of Jiangsu Province)

Shandong Provincial Center for Disease Control and Prevention

Anhui Provincial Center for Disease Control and Prevention

Hunan Provincial Center for Disease Control and Prevention

Chongqing Municipal Center for Disease Control and Prevention

Yunnan Provincial Center for Disease Control and Prevention

Version No.: 1.2, Version Date: Jan. 13, 2022

Study Title	A clinical trial of Heterologous booster immunization with recombinant COVID- 19 vaccine (adenovirus type 5 vector) for inhalation after primary series of inactivated COVID-19 vaccine		
Study Title Description	Safety and immunogenicity of one dose of booster vaccination with recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation in adults aged 18 years and above: A multicenter, open-label, partially randomized controlled clinical trial		
Project No.	JSVCT137		
Investigational Vaccine	Recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation (hereinafter referred to as Ad5-nCoV-IH) Inactivated COVID-19 Vaccine (Vero Cell) (hereinafter referred to as ICV)		
Protocol Date	Jan. 13, 2022		
Version No.	1.2		
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PRINCIPAL INVESTIGATOR'S STATEMENT

I hereby agree:

To assume the responsibilities of a principal investigator of the clinical study.

To ensure that the study is conducted in accordance with the trial protocol and amendments agreed by all related parties.

To ensure that all personnel involved in this study have a full understanding of the investigational vaccine as well as the study-related responsibilities and obligations specified in the study protocol.

To ensure that changes to the study protocol are not implemented without prior review and written approval from the ethics committee, except where necessary to eliminate an immediate hazard to the subjects or when the changes are requested by regulatory authorities (e.g., when involving the administrative management).

I fully understand the proper use of the investigational vaccine as described in the protocol, as well as other information including but not limited to: the current Investigator's Brochure or equivalent.

I am familiar with and will comply with the "Good Clinical Practice (GCP)" and all applicable regulatory requirements.

Study Title	A clinical trial of Heterologous booster immunization with recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation after primary series of inactivated COVID-19 vaccine	
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PROTOCOL REVISIONS

Serial No.	Old Version No./Version Date/Revised Part	New Version No./Version Date/Revision Description
1	1.0/Dec. 10, 2021/Sample size determination	1.1/Jan. 6, 2022/Based on the opinions of the Scientific Review Committee
2	1.0/Dec. 10, 2021/Preliminary clinical trial results	1.1/Jan. 6, 2022/Based on the opinions of the Scientific Review Committee
3	1.0/Dec. 10, 2021/Study hypotheses	1.1/Jan. 6, 2022/Based on the opinions of the Scientific Review Committee
4	1.0/Dec. 10, 2021/Coding rules	1.1/Jan. 6, 2022/Added the site number corresponding to each site
5	1.0/Dec. 10, 2021/Personnel training	1.1/Jan. 6, 2022/Based on the opinions of the Scientific Review Committee
6	1.0/Dec. 10, 2021	1.1/Jan. 6, 2022/Some formatting and sentence adjustments
7	1.1/Jan. 6, 2022	1.2/Jan. 13, 2022/Some formatting and detail description modifications
8	1.1/Jan. 6, 2022	1.2/Jan. 13, 2022/Addition of requirements for safety review at enrollment of approximately 2000 and 5000 subjects

PROTOCOL SYNOPSIS

Study Title	A clinical trial of Heterologous booster immunization with recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation after primary series of inactivated COVID-19 vaccine
Study Title Description	Safety and immunogenicity of one dose of booster vaccination with recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation in adults aged 18 years and above: A multicenter, open-label, partially randomized controlled clinical trial
Indication	Prevention of Coronavirus Disease 2019 (COVID-19) caused by SARS-CoV-2 infection
Study Population	Adults aged 18 years and above
Number of Subjects	Approximately 10420 subjects
Objectives	Primary objective: 1. To evaluate the safety of a booster dose of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation. 2. To evaluate if the immunogenicity of a booster dose of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation is superior to that of inactivated COVID-19 vaccine. Secondary objective: 1. To evaluate the persistence of immunogenicity after a booster dose of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation.
Study Site	6 study centers
Rationale	The global pandemic of COVID-19 has brought severe threat to economy and people's lives in countries around the world. Vaccination is one of the effective ways to stop this pandemic. Currently, vaccines used globally to prevent COVID-19 mainly include inactivated vaccines, viral vector vaccines, live attenuated vaccines, recombinant protein vaccines, and nucleic acid vaccines. Inactivated vaccines manufactured by Sinopharm, Sinovac, and the Institute of Medical Biology Chinese Academy of Medical Sciences are prepared by inactivating the entire SARS- CoV-2 virus, and have demonstrated good safety and efficacy. The recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation, jointly developed by Beijing Institute of Biotechnology and CanSino Biologics Inc., is prepared by amplification and purification of replication-defective adenovirus serotype 5 vectors that express the SARS- CoV-2 spike protein. Clinical trial results have demonstrated that this vaccine is safe and reliable, and can induce both humoral and cellular immunity to prevent the virus from infecting host cells and to eliminate cells infected by the virus. Recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation involves innovation of different routes of immunization on the basis of intramuscular injection, whose formulation is the same as that of the vaccine for intramuscular injection, but a different route of immunization, i.e., nebulized inhalation, is used. Preclinical and ongoing clinical studies have shown that vaccination via nebulized inhalation stimulates the mucosal immune system to produce IgA antibodies in the respiratory mucosa, including sIgA on the mucosal surface of the respiratory tract and serum IgA. At the same time, it can stimulate humoral immunity and produce serum IgG antibodies. Furthermore, it can stimulate strong cellular immunity, producing interferon IFN-γ and interleukin. Considering the differences in the types and characteristics of immune response induced by the inactivated vaccine and the recombinant COVID-19 vaccine (adenovirus type 5 vector), heterologous vaccination with these two vaccines may be complementary, improving the speed and quality of the immune response, focusing the immune response on S protein, generating higher titers of broad- spectrum neutralizing antibodies, and longer-lasting immune memory, ultimately optimizing current COVID-19 immunization strategies.

<table>
<tr><td>Investigational Vaccine</td><td>Investigational vaccine 1: inactivated COVID-19 vaccine (Vero cell) (hereinafter referred to as ICV)
Manufacturer: Sinovac Biotech Ltd./Beijing Institute of Biological Products Co., Ltd./Wuhan Institute of Biological Products Co., Ltd./Shenzhen Kangtai Biological Products Co., Ltd.
Strength: 0.5 mL/vial.
Dose: 0.5 mL
Administration route: Intramuscular injection in the lateral deltoid of the upper arm
Investigational vaccine 2: Recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation (hereinafter referred to as Ad5-nCoV-IH)
Manufacturer: Beijing Institute of Biotechnology/CanSino Biologics Inc.
Strength: 1.5 mL/vial (1.5×10^{11} VP)
Dose: 0.1 mL (1×10^{10} VP)
Administration route: Oral inhalation using a nebulizer
Storage and transportation conditions:
Store and transport at 2–8 °C, away from light and freezing.</td></tr>
<tr><td>Study Design</td><td>Study design:
Multi-center, open-label clinical study, with a randomized, controlled immunogenicity group.
Sample size determination:
This study includes a safety group and an immunogenicity group. The immunogenicity group also includes a cellular immunity group. Approximately 10,000 subjects are planned to be enrolled in the safety group. This sample size provides at least 85% power to detect 1 case of adverse reaction in the investigational vaccine group with an incidence of 0.02% (assuming that the background incidence of a given adverse event is 1 in a million).
Rationale for sample size determination in the immunogenicity group: Based on data from preliminary clinical studies (Project No.: JSVCT127), the GMT of anti-SARS-CoV-2 neutralizing antibodies at 28 days after administering a booster dose of the inactivated vaccine in the control group was around 16. The GMT, standard deviation, of anti-SARS-CoV-2 neutralizing antibodies at 28 days after immunization in the investigational vaccine group were 32, and 9, respectively. Using PASS 15.0 procedure of Two-Sample T-Tests Assuming Equal Variance, 183 subjects in each of the investigational vaccine group and the control group (1:1 ratio) will be required for a one-sided $\alpha = 0.025$ test with at least 85% power to demonstrate that the antibody titer after booster dose in the investigational vaccine group is superior to the control group. Considering the dropout of the study, 420 subjects will be enrolled in the immunogenicity subgroup. The cellular immunity group will consist of the first 60 subjects in the immunogenicity group of a single site managed by Jiangsu CDC.

Table 1. Group design and sample size
<table><tr><th>Group</th><th>Vaccine/Dose</th><th>Study Center</th><th>Sample Size (subject)</th><th>Total (subject)</th></tr><tr><td rowspan="5">Safety Group</td><td rowspan="5">Ad5-nCoV-IH
1.0×10^{10} VP
(0.1 mL)</td><td>Jiangsu CDC</td><td>2500</td><td rowspan="5">10000</td></tr><tr><td>Chongqing CDC</td><td>1500</td></tr><tr><td>Shandong CDC</td><td>1500</td></tr><tr><td>Hunan CDC</td><td>1500</td></tr><tr><td>Anhui CDC</td><td>1500</td></tr></table></td></tr>
</table>

		Yunnan CDC	1500	
Immunogenicity Group	Ad5-nCoV-IH 1.0×10^{10} VP (0.1 mL) or ICV (0.5 mL)	Jiangsu CDC	120	420
		Chongqing CDC	60	
		Shandong CDC	60	
		Hunan CDC	60	
		Anhui CDC	60	
		Yunnan CDC	60	

Notes:

1. Recruited subjects must have completed prime immunization with 2 doses of inactivated vaccine.
2. The first 60 subjects (120 subjects for Jiangsu CDC) from a single site of each center will be included in the immunogenicity group.
3. The cellular immunity group will consist of the first 60 subjects in the immunogenicity group of a single site managed by Jiangsu CDC, who will undergo ICS testing on Day 0 and 14.

Randomization:

The 420 subjects in the immunogenicity group will be randomized to receive a booster dose of Ad5-nCoV-IH or ICV in a 1:1 ratio. Authorized investigators may assign each enrolled subject a study ID via the IWRS system. The investigators responsible for managing the vaccines will use the investigational vaccine that corresponds to the study ID and group allocation information.

Study plan:

This study plans to enroll 10,420 subjects aged 18 years and old. Subjects ≥ 60 years should account for ≥ 30% of the total population. Subjects must have completed prime immunization with 2 doses of inactivated vaccine. The booster immunization must be ≥ 6 months from the second dose. The safety group will include approximately 10,000 subjects, who will receive a booster dose of Ad5-nCoV-IH to determine the safety of the vaccine.

The immunogenicity group will include 420 subjects, and subjects ≥ 60 years should account for ≥ 30% of the population. Eligible subjects who passed consultation and screening and provided informed consent will be randomized in a 1:1 ratio to receive a booster dose of either ICV or Ad5-nCoV-IH. Except for the Jiangsu CDC, the first 60 subjects from a single site of each of the other 5 centers will be included in the immunogenicity group. The first 120 subjects from a single site of the Jiangsu CDC will be included in the immunogenicity group, and the first 60 of these subjects will be included in the cellular immunity group.

Subjects assigned to receive an ICV booster dose must receive the vaccine from the same manufacturer as the two-dose prime immunization. All subjects will be issued diary cards on the day of vaccination and on Day 14 after vaccination for systematic safety observations. Venous blood and saliva samples will be collected from subjects in the immunogenicity group on the day of vaccination as well as at 14 days, 28 days, 3 months, and 6 months after vaccination to test for serum antibody titers, serum IgA, and sIgA. Venous blood samples will be collected from subjects in the cellular immunity group on the day of and at 14 days after vaccination to test for cellular immunity. All subjects are required to complete interviews on the day of vaccination as well as at 14 and 28 days after vaccination. Subjects in the immunogenicity group are also required to complete 3- and 6- month follow-up visits. The remaining subjects may complete the 3- and 6-month visits via telephone follow-up. When about 2000 subjects (first stage) and 5000 subjects (second stage) are enrolled, the safety of all subjects in the early stage should be evaluated, and the enrollment schedule should be continued with the consent of PI. Enrollment will be

	suspended or terminated immediately if a safety issue with vaccination is found to meet the suspension or termination criteria.
Study Endpoints	Primary endpoint: 1) Incidence of adverse reactions (ARs) within 0–28 days after booster immunization. 2) Geometric mean titer (GMT) of anti-SARS-CoV-2 specific neutralizing antibodies against omicron BA.4/5 in subjects in the immunogenicity group at 28 days after booster immunization. Secondary endpoints: Immunogenicity endpoints: 1) Seroconversion rate and geometric mean increase (GMI) of anti-SARS-CoV-2 neutralizing antibodies in subjects in the immunogenicity group at 28 days after immunization; 2) Seroconversion rate, GMT, and GMI of anti-SARS-CoV-2 neutralizing antibodies in subjects in the immunogenicity group at 14 days, 3 months, and 6 months after booster immunization; 3) Geometric mean concentration (GMC), seroconversion rate, and GMI of anti-SARS-CoV-2 RBD protein IgG antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after booster immunization; Safety endpoints: 1) Incidence of adverse reactions within 30 minutes after booster immunization; 2) Incidence of adverse reactions within 0–14 days after booster immunization; 3) Incidence of adverse events within 0–28 days after booster immunization; 4) Incidence of serious adverse events (SAEs) within 6 months after booster immunization. Exploratory endpoints: 1) Baseline anti-Ad5 vector neutralizing antibody titer in subjects in the immunogenicity group, and stratified analysis based on baseline anti-Ad5 vector neutralizing antibody titer ($> 1:200$, $\leq 1:200$); 2) GMT, seroconversion rate, and GMI of certain VOC/VOI cross-neutralizing antibodies in subjects in the immunogenicity group at 28 days after booster immunization; 3) GMC, seroconversion rate, and GMI of salivary IgA and serum IgA antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after booster immunization; 4) Seropositive rate and response levels of IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-13 in $CD4^+$ and $CD8^+$ T-cells stimulated by S protein overlapping peptide library using ICS at 14 days after booster immunization.

Visit Schedule

The study includes 5 visits: V1 (Day 0), V2 (Day 14), V3 (Day 28), V4 (3 months), and V5 (6 months).

V1: Informed consent, subject screening, randomization, pre-immunization blood and saliva sample collection (immunogenicity group only), immunization, observation, and dispensation of diary card (0–14 days);

V2: Adverse event observation, return of diary card (0–14 days), dispensation of diary card (after 14 days), blood and saliva sample collection (immunogenicity group only);

V3: Adverse event observation, blood and saliva sample collection (immunogenicity group only), return of diary card (after 14 days);

V4–V5: Blood and saliva sample collection (immunogenicity group only), adverse event observation

Visit point	**V1**	**V2**	**V3**	**V4**	**V5**
Visit time	D-7-D0	D14	D28	Month 3	Month 6
Time window	/	± 3 days	± 5 days	± 15 days	± 15 days
Informed consent	●				
Registration/identity verification	●	●	●	●	●
Physical examination (blood pressure, height, weight, body temperature)	●				
Urine pregnancy (female of childbearing potential)	●				
Medical history inquiry and eligibility screening	●				
Randomization number assignment[a]	●				
Immunogenicity blood sampling[a]	10 mL	10 mL	10 mL	10 mL	10 mL
Cellular immunity blood sampling[b]	15 mL	15 mL			
Saliva sampling[c]	2 mL	2 mL	2 mL	2 mL	2 mL
Immunization[d]	●				
Dispensation of diary card (0–14 days)	●				
Return of diary card (0–14 days) Dispensation of diary card (after 14 days)		●			
Return of diary card (after 14 days)			●		
Safety observations[e]	●	●	●		
SAE observation and reporting[f]	●	●	●	●	●

	a) Immunogenicity group only; b) Subjects in the immunogenicity group recruited from Jiangsu CDC who are included in cellular immunity group only; c) Immunogenicity group only; d) If the investigator believes the health status of the subject on the day of vaccination is temporarily unsuitable for vaccination, the dose may be delayed up to 1 week; e) All adverse events within 0–28 days after immunization should be observed and documented, regardless of whether related to the vaccine; f) The investigator should fill out the "Serious Adverse Event Report Form" and complete the initial report within 24 h of learning of an SAE and complete the final report at the end of the event. Except for subjects in the immunogenicity subgroup, all subjects should complete V4–V5 via telephone.
Criteria for Study Interruption And Premature Termination	The trial should be suspended, and the investigator should hold an expert panel meeting to decide whether to terminate the clinical trial prematurely if any of the following situations occur: − One subject of Grade 4 or greater adverse reactions possibly related to vaccination; − One subject of serious or life-threatening SUSAR; − The number of subjects with Grade 3 adverse reactions and symptoms lasting more than 48 h without remission to Grade 1 or Grade 2 exceeds 15% of vaccinated subjects. In case of any of the following circumstances, the study should be terminated: − The IEC requests a complete termination of trial with reasons; − The administrative department requests a complete termination of trial with reasons.
Statistical Analysis	First-stage analysis: The first analysis will be performed after the 28-day safety data have been reviewed. Second-stage analysis: The second analysis will be performed after the 28-day immunogenicity data have been reviewed. Final analysis: The final analysis will be performed after the 6-month safety and immunogenicity data have been reviewed.
Inclusion/Exclusion Criteria	Inclusion criteria: 1) Volunteers aged 18 years and above at the time of screening; 2) Volunteers who provided informed consent and signed the informed consent form; 3) Volunteers who are able and willing to comply with the requirements of the clinical trial protocol, and can complete the 6-month follow-up; 4) Completed 2 doses of vaccination with inactivated COVID-19 vaccine ≥ 6 months prior to the planned vaccination in this study. Exclusion criteria: 1) Subjects with a history or family history of convulsions, epilepsy, encephalopathy, or mental disorders; 2) Subjects who are allergic to any ingredient of the investigational vaccine, or subjects with a history of severe anaphylactic reactions to vaccines, allergies, or asthma; 3) Subjects with a history of severe vaccine-related adverse reactions following immunization with a COVID-19 vaccine;

	4) Females who tested positive for urine pregnancy test; 5) Subjects with acute febrile disorders, infectious disease, or a history of SARS; 6) Axillary temperature > 37.0 °C; 7) Subjects with serious cardiovascular diseases, such as arrhythmia, heart block, myocardial infarction, and serious, uncontrolled hypertension despite antihypertensive drugs (on-site measurement: SBP ≥ 180 mmHg and DBP ≥ 110 mmHg); 8) Subjects with serious chronic diseases or progressive diseases that cannot be controlled, such as diabetes or thyroid disorders; 9) Subjects with congenital or acquired angioedema/neurogenic edema; 10) Subjects with a history of urticaria within 1 year prior to receiving the investigational vaccine; 11) Subjects with asplenia or functional asplenia; 12) Subjects with lung function abnormalities such as chronic obstructive pulmonary disease or pulmonary fibrosis; 13) Subjects with a history of COVID-19 infection/disease; 14) Subjects with epidemiological exposure to SARS-CoV-2, or travel history to areas of moderate/high risk or abroad within the past 21 days; 15) Those who are judged by the investigator with any contradiction to the study protocol or the signing of informed consent being affected due to various medical, psychological, social, or other conditions.
Duration	Approximately 6 months
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LIST OF ABBREVIATIONS

Abbreviation	Full name
AE	Adverse Event
AR	Adverse Reaction
Ad5	Replication Defective Human Adenovirus Serotype 5
COVID-19	Corona Virus Disease 2019
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMC	Geometric Mean Concentration
GMI	Geometric Mean Fold Increase
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titre
IEC	Independent Ethics Committee
ITT	Intent-to-treat
NIFDC	National Institute for Food and Drug Control
NMPA	National Medical Products Administration
PPS	Per Protocol Set
SAE	Serious Adverse Event
SS	Safety Set
VP	Virus Particle

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 OBJECTIVES AND INTRODUCTION

SARS-CoV-2 is a non-segmented, positive-sense single-stranded RNA virus, and belongs to orthocoronavirinae subfamily of coronaviridae family in the nidovirales order. There are 6 known human infective coronaviruses, including HCoV-229E and HCoV-NL63 in α group, HCoV-OC43 and HCoV-HKU1 in β group, middle east respiratory syndrome-related coronavirus (MERS-CoV), and severe acute respiratory syndrome-related coronavirus (SARS-CoV). SARS-CoV-2 is the 7th human-infective coronavirus. Following the severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2002 and the middle east respiratory syndrome coronavirus (MERS- CoV) outbreak in 2012, SARS-CoV-2 was the third highly pathogenic coronavirus appeared in human beings in the past 20 years.

The recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation, jointly developed by Beijing Institute of Biotechnology and CanSino Biologics Inc., is a prophylactic against COVID-19 caused by SARS-CoV-2 infection. The vaccine contained replication-incompetent human adenovirus type 5 (Ad5) vectors expressing the specific S protein of SARS-CoV-2, and was prepared by amplification and purification. Preclinical studies suggested that both humoral and cellular immunity played an important role in protective immunity. The main role of humoral immunity is to prevent viral infection of host cells, while the main role of cellular immunity is to eliminate virus- infected cells.

On Mar. 16, 2020, a Phase I clinical trial of recombinant COVID-19 vaccine (adenovirus type 5 vector) was initiated in Wuhan, China, and the results showed that the low- and medium-dose vaccines showed good safety in humans, and the high-dose vaccine showed clinically tolerable safety. On Apr. 12, 2020, a phase II clinical trial of recombinant COVID-19 vaccine (adenovirus type 5 vector) was initiated in Wuhan. The results showed that low- and medium-dose vaccines exhibited good safety in humans, and the safety of low-dose vaccine was superior to that of medium-dose vaccines. Analysis of humoral immune response showed that the seroconversion rates of S- RBD protein in the low-dose group and the medium-dose group were higher. The specific IFN-γ seropositive rates in the low-dose and medium-dose groups determined by ELISpot were significantly higher than that in the placebo group [2]. On Sep. 21, 2020, a phase IIb clinical trial of recombinant COVID-19 vaccine (adenovirus type 5 vector) was initiated in Taizhou, Jiangsu to evaluate the immunogenicity and safety of 2 doses of recombinant COVID-19 vaccine (adenovirus type 5 vector) in subjects aged 6 and above who had previously received recombinant ebola virus disease vaccine (adenovirus type 5 vector). Interim results showed that 0.3 mL of the vaccine is safe in teenagers, with immunogenicity similar to that of 0.5 mL administered in adults aged 18–59 years.

A phase III clinical trial of recombinant COVID-19 vaccine for injection (adenovirus type 5 vector) was carried out in five countries including Mexico, and more than 44,000 subjects have been vaccinated. The results of the interim analysis showed that the overall protective efficacy 14 days following the first dose was 68.8%, with a protection rate of 67.8% in the elderly, and 95.5% in the critically ill; there was no vaccine-related SAE.

Therefore, the recombinant COVID-19 vaccine (adenovirus type 5 vector) has good safety and efficacy. On Feb. 25, 2021, the vaccine gained conditional marketing approval from the National Medical Products Administration; the product was also approved for emergency use by Mexico, Pakistan, Hungary, Chile, and other countries in the same time period.

Recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation involves innovation of different routes of immunization on the basis of intramuscular injection, whose formulation is the same as that of the vaccine for intramuscular injection, but a different route of immunization, i.e., nebulized inhalation, is used. Preclinical and ongoing clinical studies have shown that vaccination via nebulized inhalation stimulates the mucosal immune system to produce IgA antibodies in the respiratory mucosa, including sIgA on the mucosal surface of the respiratory tract and serum IgA. At the same time, it can stimulate humoral immunity and produce serum IgG antibodies. Furthermore, it can stimulate strong cellular immunity, producing interferon IFN-γ and interleukin. A phase I/II clinical trial of booster immunization with recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation following prime immunization with intramuscular injection of Ad5-nCoV has been carried out in China. Enrollment and vaccination of all subjects have been completed. Preliminary data showed that the booster dose was safe in people of different ages within 28 days after immunization, and immunogenicity was significantly increased.

Considering the differences in the types and characteristics of immune response induced by the inactivated vaccine and the recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation, heterologous vaccination with these two vaccines may be complementary, improving the speed and quality of the immune response and ultimately optimizing current COVID-19 immunization strategies. This study is intended to explore the safety and immunogenicity of a booster dose of Ad5-nCoV-IH or ICV at ≥ 6 months after 2 doses of inactivated COVID-19 vaccine in healthy adults 18 years and above. This clinical trial protocol is established based on "Law on Vaccine Management", "Drug Registration Regulation", GCP, "Technical Guidelines for Clinical Trials of Vaccines", "Technical Guidelines for Preclinical Research on Preventive Vaccines" and "Quality Guidelines for Clinical Trials of Vaccines" (Tentative).

2 STUDY MANAGEMENT

2.1 Study Centers

(1) To participate in the establishment of the clinical trial protocol and organize the implementation of the clinical trial protocol;

(2) To assist in the preparation and review of on-site application forms such as the informed consent form, immunization and visit record, and diary card;

(3) To submit review materials for ethics to the Independent Ethic Committee (IEC) and acquire approval;

(4) To establish vaccine clinical trial organization management system and quality management system, and conduct training;

(5) To recommend clinical study sites, to organize and assist standardized establishment of the site;

(6) To prove administrative mechanism and actions to prevent and handle emergencies during the vaccine clinical trial, and to provide specialist team for SAE emergency management and technical capability of SAE management;

(7) To organize on-site subject recruitment and enrollment, on-site vaccination, and to supervise implementation of the process;

(8) To organize and ensure safe storage and use of the investigational vaccine and perform good management of biological samples;

(9) To organize subject follow-up and collection of AEs on site, and to organize report, investigation and handing of AEs;

(10) To organize completion of all forms and electronic Case Report Forms (eCRFs) at the study site;

(11) Confirm the archiving of study documents, and manage and save the relevant study documents according to GCP requirements until 5 years after the marketing approval of the investigational product;

(12) To prepare final report of the clinical trial.

2.2 Monitoring Institution —Shanghai Stem Pharmaceutical Development Co., Ltd.

(1) To confirm that the clinical trial institutions in charge of the trial have the appropriate conditions to complete the trial, including personnel and training, complete and well- functioning functional areas (e.g., emergency room) and laboratory equipment, and various conditions related to the trial;

(2) To verify that the investigational vaccines are transported, stored, dispensed, used, returned, and handled per protocol throughout the trial, and perform control and record;

(3) To confirm that all subjects have provided written informed consent as required prior to the trial, and that the subjects enrolled are eligible;

(4) To confirm that the investigators receive the latest version of Investigator's Brochure, protocol, and all documents related to the clinical trial, and which are implemented normally per regulatory requirements;

(5) To verify that all investigators have received training and written authorization prior to study participation;

(6) To confirm correct and complete record and report of all data, correct completion and consistency to the original information of all eCRFs; to verify that all medical reports, records and documents provided by the investigators are accurate, complete, timely, clear and readable, annotated with date and trial number; to verify that all correction, addition and deletion to data are correct, annotated with date, and signed by the investigator;

(7) To confirm that all AEs are recorded, and that SAEs are reported and recorded within the time specified;

(8) To confirm that the investigators save the documents required per GCP requirements, and that the trial records and documents are updated in time and well maintained;

(9) To confirm deviations from the protocol, GCP, and applicable regulatory requirements, to communicate with the investigators, and to take appropriate actions to prevent the deviation from occurring again;

(10) The CRA should submit written reports to the study center after each monitoring visit, and specify the corrective measures taken or proposed for issues identified during the visit; truthfully record the follow-ups, trials, and tests that the investigator failed to perform, and whether errors or omissions were corrected;

(11) To assist the research institution in document preparation for submission to NMPA after completion of the clinical trial, and to assist in preparing on-site inspections for registration.

2.3 Sample Testing Institutions—Vazyme Biotech Co., Ltd. and Jiangsu Provincial Center for Disease Control and Prevention (Public Health Research Institute of Jiangsu Province)

(1) To complete the testing of clinical samples according to the specified methods and issue testing reports;

(2) To provide reference value for result determination;

(3) To provide laboratory related qualifications, including certification, accreditation, and quality control.

2.4 Data Management and Statistical Analysis Institution—Shanghai Canming Pharmaceutical Technology Co., Ltd.

(1) To participate in preparation of the clinical trial protocol;

(2) Prepare statistical analysis plan as per the clinical trial protocol;

(3) To perform randomization coding to the investigational vaccine;

(4) To take charge of project eCRF establishment and data management;

(5) To perform statistical analyses and prepare a statistical analysis report.

3 BACKGROUND

3.1 Background on Disease

The main manifestations of COVID-19 disease are fever, dry cough, and asthenia. In minor amounts of patients, the disease is associated with symptoms such as nasal congestion, rhinorrhea, pharyngalgia, myalgia, and diarrhea. For most severely ill patients, dyspnea and/or hypoxemia occur at one week after onset, and in critical cases, the disease may progress rapidly into acute respiratory distress syndrome (ARDS), septic shock, incorrigible metabolic acidosis and hemorrhage/coagulation disorder, multiple organ failure, etc. Notably, severe or critical patients may develop a mild-to-moderate fever in the course of disease, or even have no evident fever. In some children and neonates, the symptoms may be atypical and manifested as digestive symptoms such as diarrhea and vomiting, or as psychasthenia and tachypnea only. In patients with mild diseases, the only symptoms are low-grade fever, minor asthenia, etc., without pneumonia manifestations. From the perspective of existing cases, a majority of the patients have good outcomes, while a minority of the patients are critically ill. Elderly patients and patients with chronic underlying diseases have poor outcomes. Pregnant women and puerperants with COVID- 19 are similar to patients of the same age in the clinical course. The symptoms in children are milder.

The main infection source is SARS-CoV-2 infected patients. Asymptomatic patients may also become infection sources. SARS-CoV-2 are prevalent in the human and are mainly transmitted by respiratory droplets and close contact. Aerosol transmission is likely to occur when people are exposed to high-concentration aerosol in a relatively closed ambiance for a long time. As SARS-CoV-2 can be isolated from stools and urine, the aerosol or contact transmission due to environmental contamination by stools and urine should be paid attention to.

3.2 Background on Virus

COVID-2019 is an infectious disease due to infection with SARS-CoV-2. At the end of 2019, the SARS-CoV-2 was first found in the cases with viral pneumonia in Wuhan, China and the disease was named COVID-19 by the World Health Organization on Feb. 11, 2020.

SARS-CoV-2 belongs to the β group of coronavirus, with envelope; particles are round or oval, frequently polymorphic, with a diameter of 60–140 nm. The genetic characteristics are obviously distinct from those of SARS-CoV and MERS-CoV. Chinese scientists found that the nCoV-2019 genome had 88% identity to two bat-derived coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) from Zhoushan, China[3]. The SARS-CoV-2 found in Wuhan is the 7th human-infective coronavirus and had not been found previously in human beings.

In genealogical classification, SARS-CoV-2 belongs to coronavirus of coronaviridae. Such positive-sense single-stranded RNA viruses with viral envelope are widely existing in nature. Globally, 10%–30% of upper respiratory infections are caused by four coronaviruses: HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, ranking the second in the etiologies of common cold, following rhinoviruses. Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) are known as severe infectious diseases caused by coronaviruses.

The coronavirus genome successively encodes spike protein (S), envelope protein (E), membrane protein (M), and nucleoprotein (N), among which S protein is the most important surface protein in coronaviruses and is associated with the infecting capability of such viruses. S protein contains two subunits: S1 and S2, of which S1 mainly contains the receptor-binding domain and is responsible for recognizing cell receptors, and S2 contains the primary elements required in the membrane fusion process. During the previous development of SARS and MERS vaccines, S protein is regarded as the most important candidate antigen.

3.3 Vaccine Background

The recombinant COVID-19 vaccine (adenovirus type 5 vector), developed by the Beijing Institute of Biotechnology and CanSino Biologics Inc., is based on the mature recombinant replication-defective human Ad5 vector platform and can express efficiently target antigen of SARS-CoV-2 (protein S), and as expected, may be able to induce humoral immunity and cell- mediated immune responses against SARS-CoV-2 envelope protein after the vaccination, to provide protection for vaccine recipients.

Presently, the SARS-CoV-2 vaccines in development include the following:

Inactivated vaccine: Inactivated vaccine is composed of intact viruses. Its pathogenicity is lost but it still retains the whole or partial immunogenicity of the virus. After vaccination, the virus antigen may stimulate the human body to produce immune responses to achieve protective effects. The inactivated vaccine requires the following steps: Virus strains are cultivated and screened in appropriate cells to obtain a virus that is stable and may represent the antigen characteristics, and is used to establish a seed bank for subsequent large-scale production of the vaccine. The candidate vaccine is prepared by cultivation, inactivation, purification, etc.; the process is relatively simple as a conventional and classic preparation mode for a vaccine. The main obstacles are: (1) The research on pathogenesis and immunological mechanism of SARS-CoV-2 is not enough, and inactivated whole virus may carry hazardous ingredients; (2) the cultivation of live virus must be conducted under P3 level biological safety conditions at present, and the production capacity is limited.

Recombinant subunit vaccine: Recombinant subunit vaccine is prepared from effective antigens that are stimulated by the virus in the human body to produce protective immunity, with assured safety; however, the vaccine is generally of small volume and poor immunogenicity, and requires new techniques and adjuvants to increase its immunogenicity. Construction and design as well as efficacy evaluation are critical points, and the research and development cycle is quite long.

Adenovirus type 5 vector vaccine: The replication defective human adenovirus serotype 5 vaccine containing SARS-CoV-2 antigen gene may efficiently express SARS-CoV-2 target antigen in transfected/infected cells, so that the human body may produce humoral immunity and cellular immunity and can provide effective protection against disease caused by SARS-CoV-2. The vaccine contains antigen genes with the same adenovirus type 5 vector platform as the recombinant Ebola virus vaccine approved, demonstrating certain foundation for research and development.

Attenuated influenza virus vector vaccine: The vaccine is vaccinated via nasal drip and may have a certain effect on increasing the vaccination rate if successfully developed. At present, no reports on similar vaccines are available in other countries.

mRNA vaccine: mRNAs of multiple antigen sequences against critical targets of SARS-CoV-2 are synthesized *in vitro* and then delivered to the human body for translation into antigen proteins by cells *in vivo* to activate the immune system and cause specific immune responses. mRNA drugs are simply manufactured, easily transformed, and rapidly synthesized, with a lower cost. However, such drugs have the disadvantages of poor stability and too strong immunogenicity. At present, most mRNA vaccine products are in the clinical stage and no product is marketed. Of them, the vaccine with the fastest progress is mRNA-1273 of Moderna Therapeutics Inc., and the vaccine has directly entered human trials without animal experiments. Recently, Moderna announced that its SARS-CoV-2 vaccine demonstrated *in vitro* neutralizing activity against multiple newly emerged SARS-CoV-2 variants, including B.1.1.7 (first found in the UK) and B.1.351 (first found in South Africa).

3.3.1 Preclinical immunogenicity assessments (mouse model: intramuscular injection + mucosal immunization)

3.3.1.1 Specific IgG antibody test results

The results showed that Ad5-nCoV immunization by both routes of administration had favorable immunogenicity: the anti-S protein IgG antibody peaked at 28 days after vaccination by intramuscular injection and then slightly decreased; the IgG antibody peaked at 28 days after vaccination and remained stable until day 56 in the mucosal immunization group; the IgG antibody titer of high-dose groups in the mucosal immunization group was higher than that in the intramuscular injection group ($P < 0.0001$ for 42 days, $P = 0.0001$ for 56 days), and there was no significant difference in IgG antibody titer between the two routes of administration at 42 days or 56 days after vaccination in the medium-dose group and the low-dose group ($P > 0.05$).

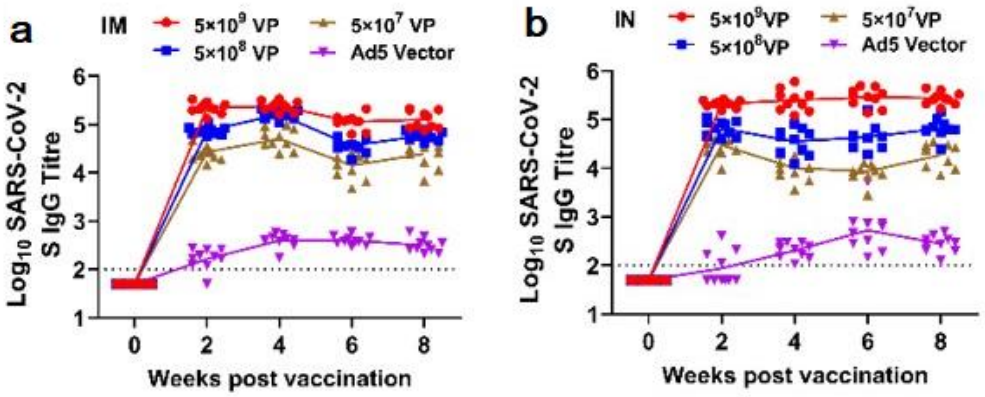


Figure 3.3-1. Plots of serum anti-S protein-specific IgG antibody levels in mice 14, 28, 42, and 56 days after single-dose immunization with different routes of administration (a. intramuscular injection; b. mucosal immunization)

Fourteen days and 10 weeks after immunization, anti-S protein-specific IgG antibodies were detected in the bronchopulmonary lavage fluid of the intramuscular injection and mucosal immunization groups, but anti-S protein-specific IgA antibodies were only detected in the mucosal immunization group, as shown in the following figure:

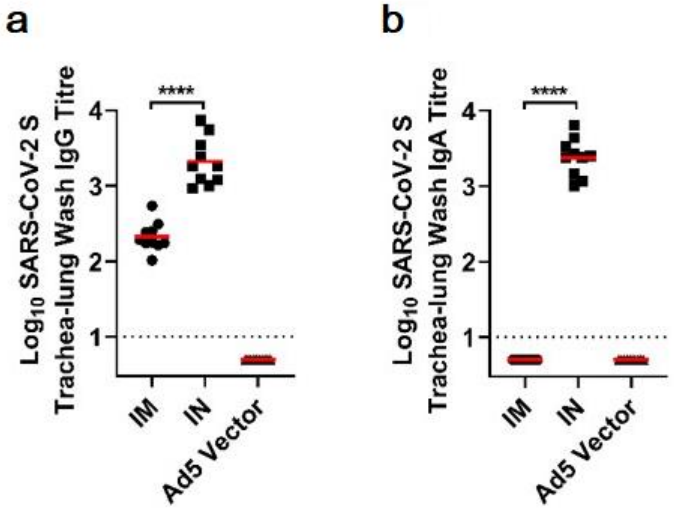


Figure 3.3-2. IgG and IgA antibody levels in lung lavage fluid of mice in the medium-dose group 14 days after single-dose immunization with different routes of administration (a. IgG titer in lung lavage fluid; b. IgA titer in lung lavage fluid; IM: intramuscular injection; IN: mucosal immunization)

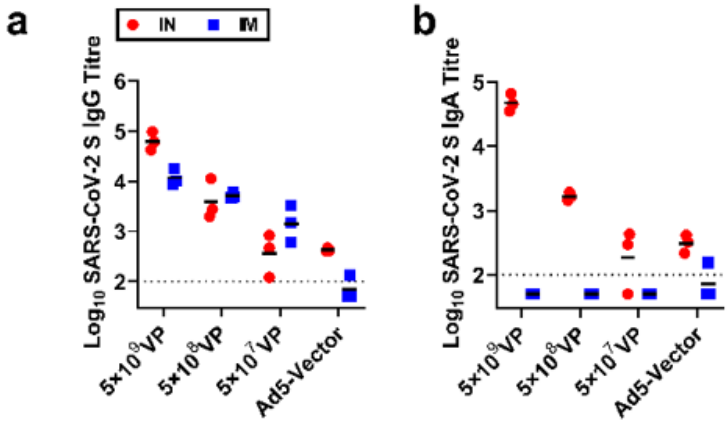


Figure 3.3-3. IgG and IgA antibody levels in lung lavage liquid of mice 10 weeks after single-dose immunization with different routes of administration (a. IgG titer in lung lavage fluid; b. IgA titer in lung lavage fluid; IM: intramuscular injection; IN: mucosal immunization)

3.3.1.2 Neutralizing antibody test results

The serum anti-SARS-CoV-2 neutralizing antibody (NAb) titers in mice 14, 28, 42 and 56 days after a single intramuscular injection and mucosal immunization were determined using virus-specific cytopathic effect-based microneutralization assay (see Figure 3.3-4 for details):

The results showed that Ad5-nCoV immunization by both routes of administration had favorable immunogenicity: the neutralizing antibody levels by mucosal immunization and intramuscular injection peaked at 42 and 56 days after vaccination, respectively: In the high-dose group, the mucosal immunization arm showed significantly higher neutralizing antibody titers at 28 to 56 days after vaccination than the intramuscular injection arm ($P < 0.0001$ for 28 and 42 days, $P = 0.0021$ for 56 days); in the medium-dose group, there was no significant difference in neutralizing antibody titers between the two routes at 42 and 56 days after vaccination; in the low-dose group, there was no significant difference in neutralizing antibody titers between the two routes of administration at each time point after vaccination.

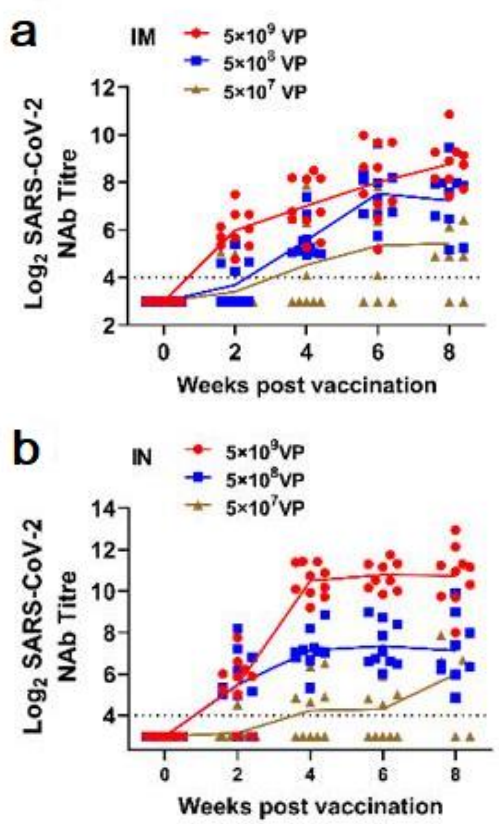


Figure 3.3-4. Plots of the serum anti-SARS-CoV-2 neutralizing antibody levels in mice at 14, 28, 42, and 56 days after single-dose immunization with different routes of administration (a. intramuscular injection; b. mucosal immunization)

The serum pseudovirus neutralizing antibody levels in mice at 14, 28, 42, and 56 days after a single intramuscular injection or mucosal immunization were tested, respectively (see Figure 3.3-5 for details). The results showed that the neutralizing antibody titer of pseudovirus was similar to that of euvirus.

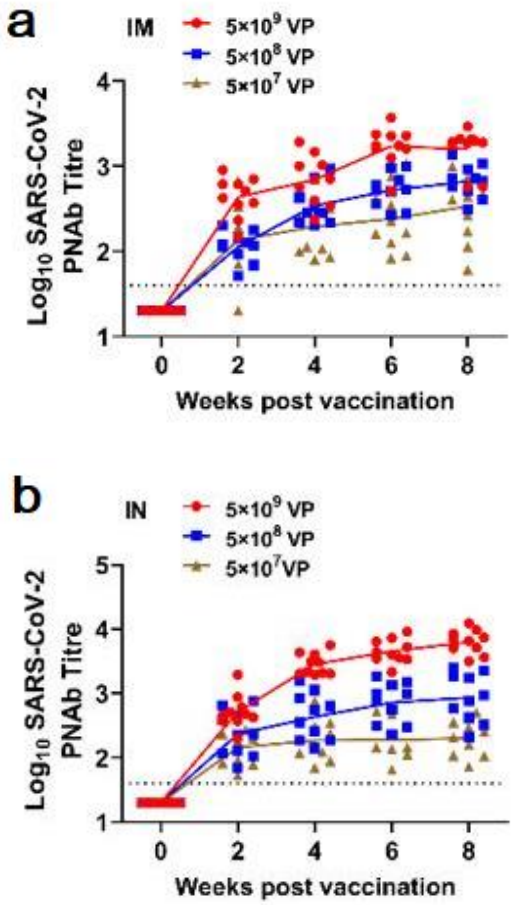


Figure 3.3-5. Plots of pseudovirus neutralizing antibody levels in mice at 14, 28, 42, and 56 days after single-dose immunization with different routes of administration (a. intramuscular injection; b. mucosal immunization)

There was a clear correlation between the IgG antibody levels, neutralizing antibody levels, and pseudovirus neutralizing antibody levels at 42 and 56 days after vaccination.

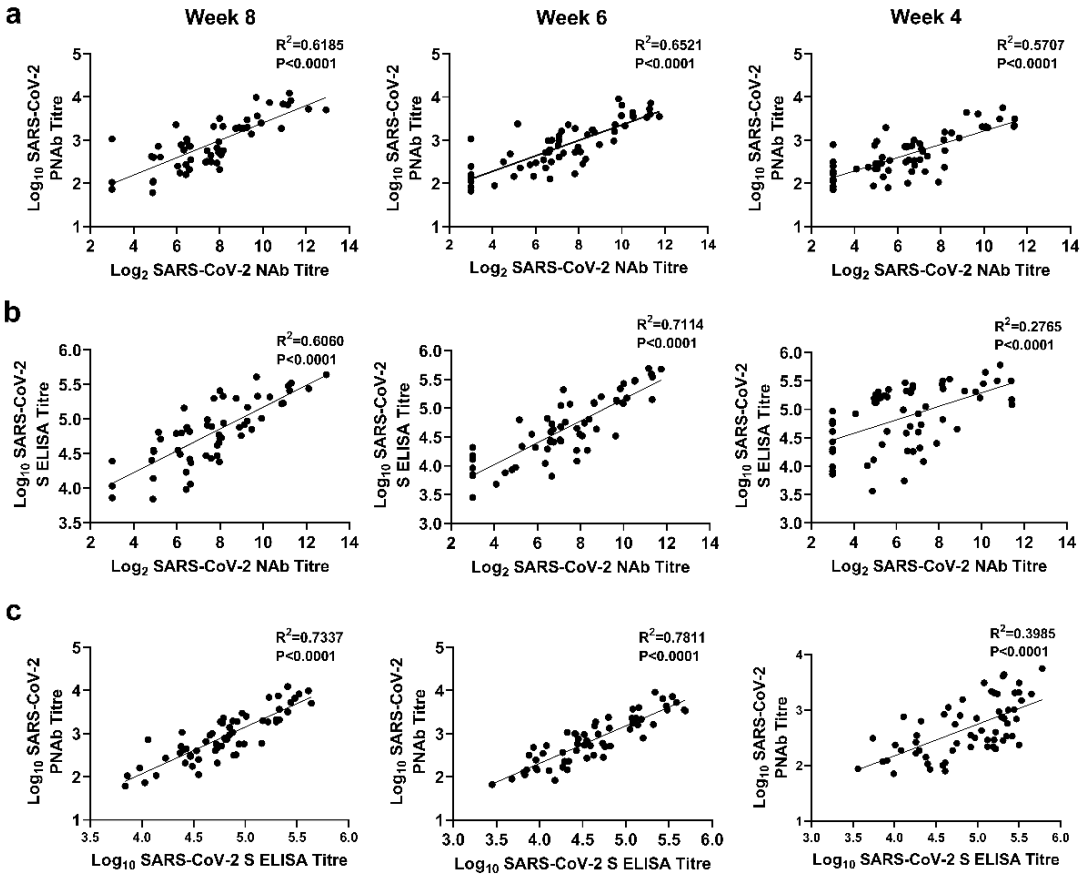


Figure 3.3-6. Correlation between the IgG antibody levels, neutralizing antibody levels, and pseudovirus neutralizing antibody levels after Ad5-nCoV immunization in mice

3.3.1.3 Cell-mediated immune response

Intracellular cytokine staining was used to determine the percentage of cytokine-positive $CD8^+$ or $CD4^+$ T cells.

Both intramuscular injection and mucosal immunization significantly induced IFNγ, TNFα, and IL-2 production by $CD8^+$ and $CD4^+$ T cells at 14 days after immunization in the medium-dose group, which was higher in the intramuscular injection group than in the mucosal immunization group.

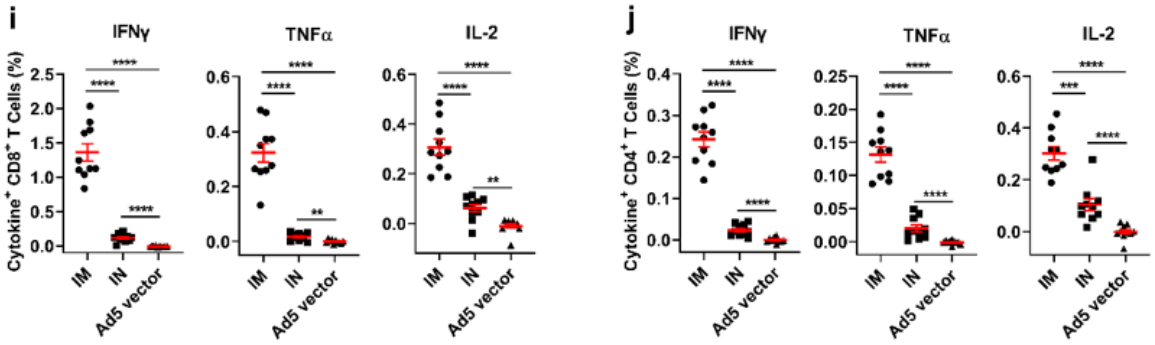


Figure 3.3-6. Cell-mediated immune response in mice at 14 days after single-dose immunization

At 10 weeks after immunization, the intramuscular injection group showed a dose-dependent increase in cell-mediated immune response, while no significant dose-dependent relationship was observed in the mucosal immunization group.

3.3.2 Preclinical protection test

3.3.2.1 Ferret model (intramuscular injection + mucosal immunization)

3.3.2.1.1 Test results of specific IgG antibodies and neutralizing antibodies

Eighteen ferrets were randomly selected and assigned to 3 groups: 6 ferrets each in the intramuscular injection, nasal drop, and negative control groups. All ferrets in the vaccination group developed S protein-specific IgG antibodies and neutralizing antibodies at 28 days after immunization, which were not detected in the control group and were not significantly different between the two routes of administration.

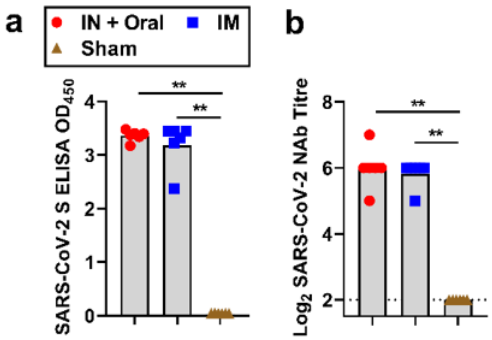


Figure 3.3-7. Results of S protein-specific IgG antibody and neutralizing antibody in ferrets

3.3.2.1.2 Cell-mediated immune response

Cell-mediated immune responses at 28 days after immunization detected by IFNγ and ELISpot assays were found in 5 and 3 ferrets in the intramuscular injection group and the mucosal immunization group, respectively.

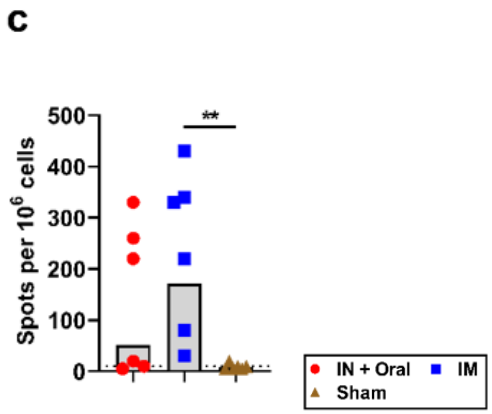


Figure 3.3-8. Cell-mediated immune response in ferrets

3.3.2.2 Rhesus monkey protective experiment (nebulized inhalation)

3.3.2.2.1. Immunogenicity at different doses

In the "Immunogenicity Study of Different Doses and Different Injections of Nebulized Immunization in Rhesus Monkeys" commissioned to Beijing JOINN Laboratories Inc., the nebulized immunization was consistent with the clinically intended nebulized inhalation route. The immunization doses were 1/2 the human dose (2.5×10^{10} VP) and 3 times the human dose (15×10^{10} VP), respectively, with two doses of nebulized immunization at 2-week intervals. Blood samples were collected before and after each immunization to determine the serum antibody level. The serum antibody levels after the first and second immunizations were determined by ELISA, and statistically analyzed by the JMP software. The results showed that the antibody level following nebulized inhalation of 3 HD in rhesus monkeys was higher than that of 1/2 HD after 1 and 2 doses of immunizations.

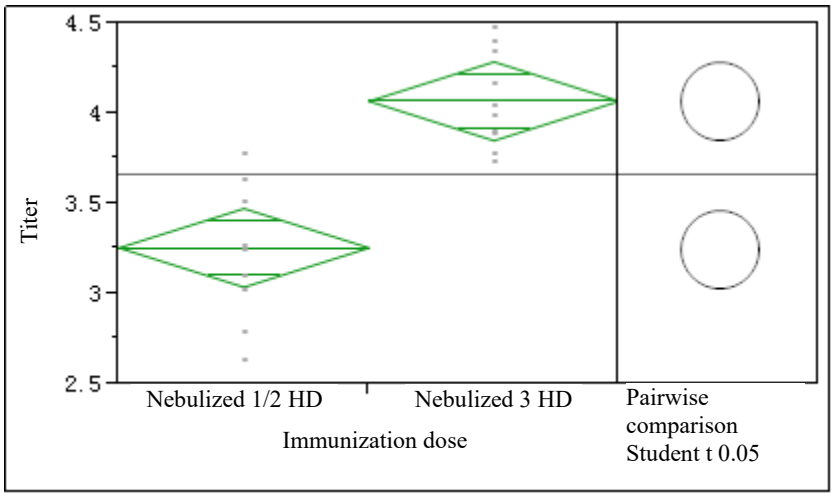


Figure 3.3-9. Serum antibody titers after the first dose of nebulized immunization at different doses

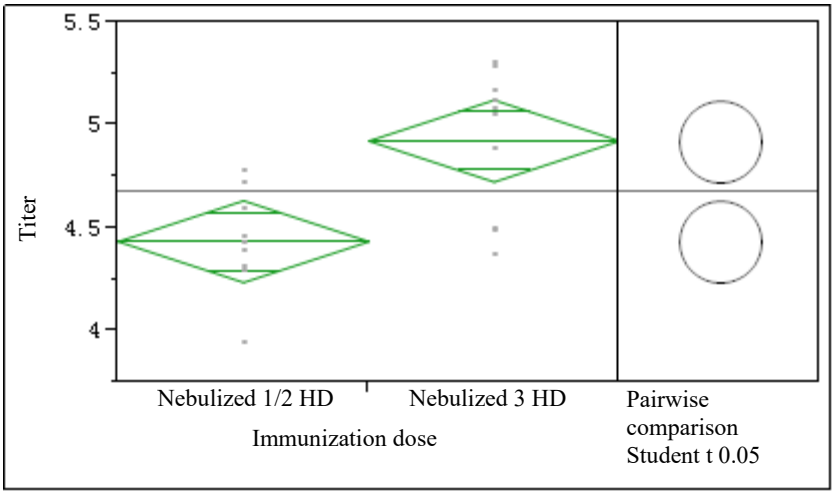


Figure 3.3-10. Serum antibody titers after the second dose of nebulized immunization at different doses

3.3.2.2.1 Immunogenicity at different injections

Nebulized inhalation of 1/2 HD test article was performed with 3 immunizations at 2-week intervals, and blood samples were collected before and after each immunization to determine the serum antibody level. The serum antibody levels after the first, second, and third immunizations were determined by ELISA. The results showed that the serum antibody levels following 2 doses of nebulized immunization were significantly higher than those of 1 dose, with statistically significant difference, but there was no significant difference between 3 and 2 doses of immunization.

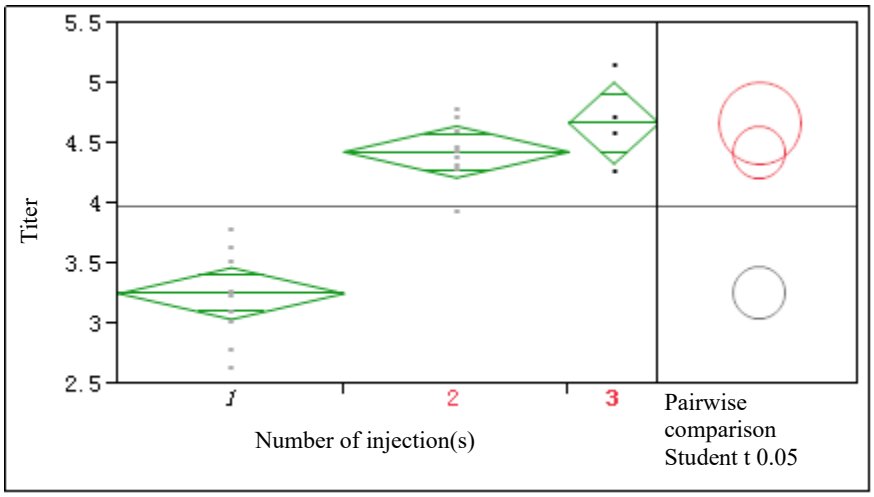


Figure 3.3-11. Serum antibody titers at different injections in rhesus monkeys

3.3.3 Preclinical safety evaluation

3.3.3.1 Single-dose toxicity study in SD rats (mucosal immunization)

Under the conditions of this study, after SD rats were given multiple doses (0.5×10^{11} VP/animal) of the recombinant COVID-19 vaccine (adenovirus type 5 vector) by nasal drops at 1 dose/animal, no toxicity was observed. The maximum tolerated dose (MTD) was greater than or equal to 0.5×10^{11} VP/animal (1 dose/animal). Anti-S antigen-specific IgG antibodies were detected in all animals at 2 weeks after administration, but no specific IgG antibodies against the adenovirus type 5 vector were detected.

3.3.3.2 Repeated-dose toxicity study in rhesus monkeys (mucosal immunization and nebulized inhalation)

Toxicity study of 4-week repeated nebulized inhalation or nasal spray of the recombinant COVID-19 vaccine (adenovirus type 5 vector) in rhesus monkeys followed by a 2-week recovery period:

During the study, no death or moribundity occurred in the animals of each group; no dosing-related abnormal response was seen during clinical observation. No anaphylactic reaction symptoms occurred in animals during clinical observation after each of the 3 doses. Compared with the animals of the same sex in the negative control group in the same period, those receiving the test article by different routes of administration had no obvious changes or abnormal changes of toxicological significance in such indexes as body weight and weight gain, body temperature, ophthalmic examination, clinical pathology (blood count, coagulation function, blood biochemistry, and urinalysis), T lymphocyte subsets ($CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^+CD4^+/CD3^+CD8^+$), serum cytokines (IL-2, IL-4, IL-5, IL-6, TNF-α, and IFN-γ), C-reactive protein, serum complements (C3 and C4), organ weight, organ-to-body ratio, and organ-to-brain ratio.

Before and after the first dose and the last dose, animals in each group showed no obvious abnormal changes or obvious changes of toxicological significance in safety pharmacological indicators including ECG waveform and parameters, blood pressure (systolic blood pressure, diastolic blood pressure, and mean blood pressure), and respiratory function (respiratory rate and tidal volume).

The immunogenicity test results showed that 10/10 animals in the negative control group were tested negative for the specific IgG antibody against S antigen, and only 1/10 animal had transient specific IgG antibody against adenovirus type 5 vector on D14. In the low and high-dose groups receiving nebulized inhalation and high-dose group receiving nasal spray, only some animals produced weak specific IgG antibodies against adenovirus type 5 vectors 2 weeks after the first administration (D14), with the antibody titer ranging from 1:100–1:200. By the end of the recovery period (D43), there were no obvious changes in the seropositive rate and titer (1:100–1:400) of specific IgG antibodies against the adenovirus type 5 vector. In the low and high-dose groups receiving nebulized inhalation and high-dose group receiving nasal spray, all animals produced specific IgG antibodies against S antigen 2 weeks after the first administration (D14), with a seropositive rate of 10/10. With the increase of the number of administrations, the range of antibody titer showed an increasing trend. Before the last administration (D28), the antibody titer of animals treated by nebulized inhalation ranged from 8721.886 to 201992.795, and that of animals treated by nasal spray ranged from 579.049 to 10487.362. Two weeks after drug discontinuation, the antibody titers in all groups were not decreased. The antibody titer of animals treated by nebulized inhalation ranged from 18154.480 to 227215.309 and that of animals treated by nasal spray ranged from 1051.386 to 15979.091.

The results of the antinuclear antibody assay showed that no antinuclear antibody was detected in animals of the negative control group and the test article groups with different routes of administration in the assay.

The results of pathological examination showed that there were no macroscopic or histopathological changes related to the test article in all groups at the end of the administration (D32) and recovery period (D43). No irritation reactions were observed at the administration sites.

Conclusion: Under the conditions of this study, the recombinant COVID-19 vaccine (adenovirus type 5 vector) was repeatedly administered by nebulized inhalation at 2.5×10^{10} VP/animal and 1.5×10^{11} VP/animal or by nasal spray at 1×10^{11}VP/animal to rhesus monkeys once every 2 weeks for a total of 3 doses, and no toxicity was observed in each group during the study. Therefore, the no-observed-adverse-effect level (NOAEL) for nebulized inhalation and nasal spray was 1.5×10^{11}VP/animal and 1×10^{11}VP/animal, respectively. Two weeks after administration (D14), all animals produced strong specific IgG antibodies against S antigen, and the antibody titer increased with the increase of administration times; no immunotoxic reactions were observed, and no irritation reactions were observed at the administration sites.

3.3.3.3 Repeated-dose toxicity study in cynomolgus monkeys (intramuscular injection)

Toxicity study of 2-week repeated intramuscular injection in cynomolgus monkeys followed by a 2-week recovery period:

During the study, no death or moribundity occurred in the animals of each group; no dosing-related abnormal response was seen during clinical observation. No anaphylactic reaction symptoms occurred in animals during clinical observation after each of the 2 doses. During the study, compared with animals of the same sex in the negative control group in the same period, no obvious change of toxicological significance was noted in various parameters in animals in the low- and high-dose groups (1 dose/animal, 3 doses/animal), including body weight and weight gain, body temperature, ECG waveform and parameters, blood pressure, ophthalmic examination, clinical pathology (blood count, coagulation function, blood biochemistry, and urinalysis), T lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$), serum cytokines (IL-2, IL-4, IL-5, IL- 6, TNF-α, and IFN-γ), C-reactive protein, serum complements (C3 and C4), organ weight, organ-to-body ratio, and organ-to-brain ratio.

3.4 Results of Preliminary Clinical Studies

3.4.1 Phase I/II clinical trials on nebulized inhalation

On Apr. 20, 2021, a randomized, double-blind, placebo-controlled phase I/II clinical trial was carried out in Donghai County, Jiangsu to evaluate the safety and immunogenicity of different doses of recombinant COVID-19 vaccine via nebulized inhalation in people aged 18 years and above.

A randomized, double-blind, placebo-controlled design was used for the phase I and phase II trials. In phase I, there were 120 subjects that were divided into 5 groups (low-dose inhalation, medium-dose inhalation, high-dose inhalation, inhalation/intramuscular injection mixed, and single-dose inhalation groups). There were 24 subjects per group and the ratio of the investigational vaccine to the placebo was 3:1. In phase II, there were 720 subjects that were divided into 2 age groups (18–59 years and ≥ 60 years) and there were 6 groups per age group (low-dose inhalation, medium-dose inhalation, high- dose inhalation, inhalation/intramuscular injection mixed, single-dose intramuscular injection, and single-dose inhalation groups). There were 60 subjects per group and the ratio of the investigational vaccine to the placebo was 5:1. An interval of 2 months (56 days) between two doses was used in the mixed nebulized inhalation group.

Preliminary safety data showed:

Overall, there were significantly fewer ARs in the low-, medium-, and high-dose arms of the nebulized inhalation group compared with the intramuscular injection group when a 2-month interval between two doses was used. The safety of the mixed group was particularly pronounced as the total incidence of AR was less than 5% after the second dose.

The preliminary safety data from the phase I trial showed that: During immunization with the first dose, the nebulized inhalation group showed good safety that was similar to the placebo group and better than the intramuscular injection group (i.e., first dose in the mixed group). One grade 3 AR (fever) occurred in the intramuscular injection group. After immunization with the second dose, incidences of ARs in the nebulized inhalation group and the mixed group (second dose: nebulized inhalation) were comparable to the placebo group, and no grade 3 ARs or vaccination-related SAEs occurred.

In the phase II clinical trial, the overall incidence of ARs 14 days after immunization in subjects between 18–59 years old in the two low-dose groups, two medium-dose groups, two high-dose groups, two mixed-dose groups, intramuscular single-dose group, medium single-dose group, and placebo group was 17 (34.00%), 15 (31.25%), 12 (24.00%), 23 (46.00%), 22 (44.00%), 5 (10.00%), and 13 (21.67%), respectively ($P < 0.001$). The incidences of grade 1 ARs in various groups were 16 (32.00%), 13 (27.08%), 10 (20.00%), 21 (42.00%), 21 (42.00%), 5 (10.00%), and 12 (20.00%), respectively ($P = 0.001$); the incidences of grade 2 ARs were 2 (4.00%), 3 (6.25%), 1 (2.00%), 4 (8.00%), 3 (6.00%), 0.00%, and 2 (3.33%), respectively ($P = 0.467$); the incidences of grade 3 ARs were 1 (2.00%), 1 (2.08%), 4 (8.00%), 0.00%, 0.00%, 0.00%, and 0.00%, respectively ($P = 0.015$). Inter-group differences in the incidences of overall ARs, grade 1 ARs, and grade 3 ARs within days 0–14 were statistically significant, while those of grade 2 ARs were not.

In the phase II clinical trial, the overall incidence of ARs 14 days after immunization in subjects aged ≥ 60 years old in the two low-dose groups, two medium-dose groups, two high-dose groups, two mixed-dose groups, intramuscular single-dose group, medium single-dose group, and placebo group was 9 (18.37%), 2 (3.92%), 3 (6.00%), 1 (2.04%), 7 (14.00%), 2 (4.00%), and 5 (8.33%), respectively ($P = 0.026$). The incidences of grade 1 ARs in various groups were 9 (18.37%), 2 (3.92%), 3 (6.00%), 1 (2.04%), 7 (14.00%), 2 (4.00%), and 4 (6.67%), respectively ($P = 0.021$); the incidences of grade 2 ARs were 2 (4.08%), 0.00%, 1 (2.00%), 0.00%, 0.00%, 1 (2.00%), and 1 (1.67%), respectively ($P = 0.544$); no grade 3 or greater ARs occurred. Inter-group differences in the incidences of overall ARs and grade 1 ARs within days 0–14 were statistically significant, while those of grade 2 ARs were not.

According to the preliminary safety observation results in the phase II clinical trial, all dose/ immunization procedure groups showed adequate safety profiles. Most ARs were grade 1 reactions and occurred within days 0–7 after each dose. There were few local ARs, and systemic ARs were mainly pyrexia, fatigue, headache, and diarrhea. The incidence of post-immunization ARs among subjects aged 18–59 years was higher than that among subjects aged ≥ 60 years.

Preliminary immunogenicity results showed:

Existing phase I and phase II immunogenicity data were combined for analysis. The 28-day data after 2 doses showed that: the mixed group showed the best result in terms of euvirus neutralizing antibody level, which was around 17 times that of the single-dose intramuscular injection group; followed by the high-dose nebulized inhalation group, which was around 8 times that of the single-dose intramuscular injection group. The euvirus neutralizing antibody level of the low- and medium-dose nebulized inhalation groups were comparable and was 2 times that of the intramuscular injection group. The mixed group and high-dose nebulized inhalation group had similar serum IgA antibody levels, which were on average higher than the low- and medium-dose nebulized inhalation groups.

An analysis of cellular immunity levels in the phase I trial was carried out. Results showed that effective cellular immunity could be generated 14 days after the first dose in various groups and higher level of cellular immunity could be induced 14 days after the second dose. The IFN-γ seroconversion rate was 80% and above and inter-group differences were not significant.

Following a single medium dose of the investigational vaccine via nebulized inhalation or two low/medium doses via nebulized inhalation, the resulting antibody levels in subjects aged 18–59 years were generally comparable to those in subjects aged ≥ 60 years. However, following two high doses via nebulized inhalation or two doses via a mixed route, the resulting antibody levels in subjects aged 18–59 years were higher than those in subjects aged ≥ 60 years.

3.4.2 A Heterologous clinical study on nebulized inhalation

In August 2021, a single-center, randomized, open-label, parallel-controlled clinical study was carried out in Donghai County, Jiangsu to evaluate the safety and immunogenicity of different doses of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation in people aged 18 years and above.

A total of 420 subjects aged 18 years and above were required to be enrolled in this study. Eligible subjects who provided informed consent and passed screening were randomly assigned at a ratio of 1:1:1 to Heterologous low-dose booster immunization group (group A), Heterologous high-dose immunization group (group B), and routine booster immunization group (group C). Subjects in groups A, B, and C must have completed prime immunization with 2 doses of the inactivated SARS-CoV-2 vaccine. Subjects enrolled in the study received a booster dose with either the inactivated SARS-CoV-2 vaccine or recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation at 3–9 months after the aforementioned 2-dose vaccinations. The first 40 subjects in each group were included in the immunogenicity subgroup for immunogenicity monitoring.

Preliminary results:

Booster immunization showed good safety in all groups, and most ARs were of grade 1. The main local ARs in the nebulized inhalation group included dysphonia, dry mouth, oral mucositis, and pharyngeal swelling, while those in the intramuscular injection group (group C) included pain, swelling, and pruritus. The incidences of local ARs in the nebulized inhalation groups were lower than those in the intramuscular injection groups. Main systemic ARs reported by all groups were pyrexia, fatigue, and headache, and inter-group differences were not statistically significant.

Fourteen days after booster immunization, the incidences of overall solicited ARs in groups A, B, and C were 26 (18.57%), 33 (23.57%), and 54 (38.57%), respectively, with inter-group differences statistically significant (***P = 0.0004***); the incidences of grade 3 ARs were 2 (1.43%), 2 (1.43%), and 0 (0.00), with $P = 0.5524$.

The incidences of solicited local ARs were 9 (6.43%), 16 (11.43%), and 45 (32.14%), respectively, for groups A, B, and C, with statistically significant differences between groups (***P < 0.0001***). Among them, the incidences of grade 1 ARs were 8 (5.71%), 16 (11.43%), and 45 (32.14%), respectively, with statistically significant differences between groups (***P < 0.0001***). The incidence of grade 2 ARs were 1 (0.71%), 2 (1.43%), and 1 (0.71%), respectively ($P > 0.9999$). No grade 3 or greater solicited local ARs occurred.

The incidences of solicited systemic reactions in groups A, B, and C were 26 (18.57%), 27 (19.29%), and 21 (15.00%), respectively ($P = 0.6014$). Among them, the incidences of grade 1 ARs were 24 (17.14%), 25 (17.86%), and 21 (15.00%), respectively ($P = 0.8002$). The incidences of grade 2 ARs were 7 (5.00%), 6 (4.29%), and 2 (1.43%) ($P = 0. 2341$). The incidences of grade 3 ARs were 2 (1.43%), 2 (1.43%), and 0 (0.00%) ($P = 0. 5524$).

After the subjects received 1 booster dose of the recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation within 3–9 months after 2 doses of inactivated vaccine, the induced levels of anti-SARS-CoV-2 S protein-RBD IgG antibodies and anti-SARS-CoV-2-specific neutralizing antibodies (euvirus) were obviously higher than those receiving 1 booster dose of the inactivated SARS-CoV-2 vaccine (intramuscular injection).

4 VACCINE OVERVIEW

Both recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation and inactivated SARS-CoV-2 vaccine are administered in a 1-dose immunization procedure with an interval of ≥ 6 months from the second dose of inactivated vaccine.

Investigational vaccine 1: inactivated COVID-19 vaccine (Vero cell) (hereinafter referred to as ICV)

Manufacturer: Sinovac Biotech Ltd./Beijing Institute of Biological Products Co., Ltd./Wuhan Institute of Biological Products Co., Ltd./Shenzhen Kangtai Biological Products Co., Ltd.

Strength: 0.5 mL/vial.

Dose: 0.5 mL

Administration route: Intramuscular injection in the lateral deltoid of the upper arm

Investigational vaccine 2: Recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation (hereinafter referred to as Ad5-nCoV-IH)

Manufacturer: Beijing Institute of Biotechnology/CanSino Biologics Inc.

Strength: 1.5 mL/vial (1.5×10^{11} VP)

Dose: 0.1 mL (1×10^{10} VP)

Administration route: Oral inhalation using a nebulizer

Storage and transportation conditions:

Store and transport at 2–8 °C, away from light and freezing.

5. STUDY OBJECTIVES

5.1 Primary Objectives

1. To evaluate the safety of a booster dose of inactivated SARS-CoV-2 vaccine or recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation.
2. To evaluate if the immunogenicity of a booster dose of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation is superior to that of inactivated COVID-19 vaccine.

5.2 Secondary Objective

To evaluate the persistence of immunogenicity after a booster dose of recombinant COVID-19 vaccine (adenovirus type 5 vector) for inhalation

6. STUDY DESIGN

Study design:

Multi-center, open-label clinical study, with a randomized, controlled immunogenicity group.

Randomization:

A total of 420 subjects in the immunogenicity group will be randomized in a 1:1 ratio to receive a booster dose of Ad5-nCoV-IH or ICV. Authorized investigators may assign each enrolled subject a study ID via the IWRS system. The investigators responsible for managing the vaccines will use the investigational vaccine that corresponds to the study ID and group allocation information.

Study design:

This study intends to enroll 10420 subjects aged 18 years and above, of which the population aged 60 years and above accounts for approximately ≥ 30%. This study includes a safety group and an immunogenicity group. The immunogenicity group also includes a cellular immunity group.

The immunogenicity group will include 420 subjects, and subjects ≥ 60 years should account for ≥ 30% of the population. Eligible subjects who passed consultation and screening and provided informed consent will be randomized in a 1:1 ratio to receive a booster dose of either ICV or Ad5- nCoV-IH. Except for the Jiangsu CDC, the first 60 subjects from a single site of each of the other 5 centers will be included in the immunogenicity group. The first 120 subjects from a single site of the Jiangsu CDC will be included in the immunogenicity group, and the first 60 of these subjects will be included in the cellular immunity group.

Subjects assigned to receive an ICV booster dose will receive the vaccine from the same manufacturer as the two-dose prime immunization. All subjects will be issued diary cards on the day of vaccination and on Day 14 after vaccination for systematic safety observations. Venous blood and saliva samples will be collected from subjects in the immunogenicity group on the day of vaccination as well as 14 days, 28 days, 3 months, and 6 months after vaccination to test for serum antibody titers, serum IgA, and SIgA. Venous blood samples will be collected from subjects in the cellular immunity group on the day of vaccination and 14 days after vaccination to test for cellular immunity. All subjects are required to complete interviews on the day of vaccination as well as 14 and 28 days after vaccination. Subjects in the immunogenicity group are also required to complete 3- and 6-month follow-up visits. The remaining subjects may complete the 3- and 6-month visits via telephone follow-up. When about 2000 subjects (first stage) and 5000 subjects (second stage) are enrolled, the safety of all subjects in the early stage should be evaluated, and the enrollment schedule should be continued with the consent of PI. Enrollment will be suspended

or terminated immediately if a safety issue with vaccination is found to meet the suspension or termination criteria.

6.1 Study Endpoints

6.1.1 Primary endpoints

1) Incidence of ARs within 0–28 days after booster immunization.

2) Geometric mean titer (GMT) of anti-SARS-CoV-2 specific neutralizing antibodies against omicron BA.4/5 in subjects in the immunogenicity group at 28 days after booster immunization.

6.1.2 Secondary endpoints

Immunogenicity endpoints:

1) Seroconversion rate and geometric mean increase (GMI) of anti-SARS-CoV-2 neutralizing antibodies in subjects in the immunogenicity group at 28 days after immunization;

2) Seroconversion rate, GMT, and GMI of anti-SARS-CoV-2 neutralizing antibodies in subjects in the immunogenicity group at 14 days, 3 months, and 6 months after booster immunization;

3) Geometric mean concentration (GMC), seroconversion rate, and GMI of anti-SARS-CoV-2 RBD protein IgG antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after booster immunization.

Safety endpoints:

1) Incidence of adverse reactions within 30 minutes after booster immunization;

2) Incidence of adverse reactions within 0–14 days after booster immunization;

3) Incidence of adverse events within 0–28 days after booster immunization;

4) Incidence of serious adverse events (SAEs) within 6 months after booster immunization.

Exploratory endpoints:

1) Baseline anti-Ad5 vector neutralizing antibody titer in subjects in the immunogenicity group, and stratified analysis based on baseline anti-Ad5 vector neutralizing antibody titer (> 1:200, ≤ 1:200);

2) GMT, seroconversion rate, and GMI of certain VOC/VOI cross-neutralizing antibodies in subjects in the immunogenicity group at 28 days after booster immunization;

3) GMC, seroconversion rate, and GMI of salivary IgA and serum IgA antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after booster immunization;

4) Seropositive rate and response levels of IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-13 in $CD4^+$ and $CD8^+$ stimulated by S protein overlapping peptide library using ICS in subjects in the cellular immunization group at 14 days after booster immunization.

6.2 Sample Size Estimation

This study includes a safety group and an immunogenicity group. The immunogenicity group also includes a cellular immunity group. Approximately 10,000 subjects are planned to be enrolled in the safety group. This sample size provides at least 85% power to detect 1 case of adverse reaction in the investigational vaccine group with an incidence of 0.02% (assuming that the background incidence of a given adverse event is 1 in a million).

Rationale for sample size determination in the immunogenicity group: Based on data from preliminary clinical studies (Project No.: JSVCT127), the GMT of anti-SARS-CoV-2 neutralizing antibodies at 28 days after administering a booster dose of the inactivated vaccine in the control group was around 16. The GMT, standard deviation, of anti-SARS-CoV-2 neutralizing antibodies at 28 days after immunization in the investigational vaccine group were 32, and 9, respectively. Using PASS 15.0 procedure of Two-Sample T-Tests Assuming Equal Variance, 183 subjects in each of the investigational vaccine group and the control group (1:1 ratio) will be required for a one-sided $\alpha = 0.025$ test with at least 85% power to demonstrate that the antibody titer after booster dose in the investigational vaccine group is superior to the control group. Considering the dropout of the study, 420 subjects will be enrolled in the immunogenicity subgroup. The cellular immunity group will consist of the first 60 subjects in the immunogenicity group of a single site managed by Jiangsu CDC.

6.3 Coding Rules

6.3.1 Screening number

After registration at the study site, each subject is given a unique screening number. Each screening number corresponds to only one subject. Rule for screening number at each site: S + site number + 4 digits.

6.3.2 Randomization numbering rules

Before vaccination during V1, each subject is assigned a unique randomization number by the study personnel on site via the IWRS system according to their enrollment sequence. Each randomization number corresponds to only one subject until the end of the study.

This study intends to enroll 10420 subjects aged 18 years and above, of which the population aged 60 years and above accounts for approximately ≥ 30%. A total of 6 study centers will be established (each study site may set up multiple vaccination sites). The 420 subjects in the immunogenicity group will be randomized to receive a booster dose of Ad5-nCoV-IH or inactivated SARS-CoV-2 vaccine in a 1:1 ratio. Rule for randomization numbers: Site number + 4-digit serial number, each site number will start with 0001. Jiangsu CDC + 0001–0060 will also be included in the cellular immunity subgroup. In the case of a subject dropping out after randomization but before vaccination, a substitute may be randomized. For example, if subject 0001 drops out before vaccination, the next enrolled subject will be the substitute with a randomization number of 10001; if the substitute also drops out before vaccination, the next enrolled subject will be a substitute with a randomization number of 20001; 9 substitutes is the maximum.

6.4 Study Duration

Expected start time: Jan. 2022.

Expected end time: Jun. 2023.

6.5 Study Interruption and Premature Termination

The investigator collects AEs of subjects after immunization. In the case of violations against the protocol, GCP requirements, or ethical requirements, the PI, IEC, or administrative departments have the right to suspend or terminate the study, and have the obligation to notify other parties and subjects and explain the reasons.

The trial should be suspended, and the investigator should hold an expert panel meeting to decide whether to terminate the clinical trial prematurely if any of the following situations occur:

- One subject of Grade 4 or greater adverse reactions possibly related to vaccination;
- One subject of serious or life-threatening SUSAR;
- The number of subjects with Grade 3 adverse reactions and symptoms lasting more than 48 h without remission to Grade 1 or Grade 2 exceeds 15% of vaccinated subjects.

In case of any of the following circumstances, the study should be terminated:

- The IEC requests a complete termination of trial with reasons;
- The administrative department requests a complete termination of trial with reasons.

7 STUDY POPULATION

7.1 Selection of Study Population

Subjects aged 18 years and above who have received two doses of inactivated SARS-CoV-2 vaccine ≥ 6 months from the booster immunization will be selected on the principle of informed consent from volunteers and voluntary participation. Among them, subjects ≥ 60 years should account for ≥ 30% of the population.

7.2 Inclusion Criteria

1) Volunteers aged 18 years and above at the time of screening;
2) Volunteers who provided informed consent and signed the informed consent form;
3) Volunteers who are able and willing to comply with the requirements of the clinical trial protocol, and can complete the 6-month follow-up;
4) Completed 2 doses of vaccination with inactivated SARS-CoV-2 vaccine ≥ 6 months prior to the planned vaccination in this study.

7.3 Exclusion Criteria

1) Subjects with a history or family history of convulsions, epilepsy, encephalopathy, or mental disorders;
2) Subjects who are allergic to any ingredient of the investigational vaccine, or subjects with a history of severe anaphylactic reactions to vaccines, allergies, or asthma;
3) Those with a history of severe vaccine-related ARs following immunization with a vaccine;
4) Females who tested positive for urine pregnancy test;
5) Subjects with acute febrile disorders, infectious disease, or a history of SARS;
6) Axillary temperature > 37.0 °C;
7) Subjects with serious cardiovascular diseases, such as arrhythmia, heart block, myocardial infarction, and serious, uncontrolled hypertension despite antihypertensive drugs (on-site measurement: SBP ≥ 180 mmHg and DBP ≥ 110 mmHg);
8) Subjects with serious chronic diseases or progressive diseases that cannot be controlled, such as diabetes or thyroid disorders;
9) Subjects with congenital or acquired angioedema/neurogenic edema;
10) Subjects with a history of urticaria within 1 year prior to receiving the investigational vaccine;

11) Subjects with asplenia or functional asplenia;

12) Subjects with lung function abnormalities such as chronic obstructive pulmonary disease or pulmonary fibrosis;

13) Subjects with a history of COVID-19 infection/disease;

14) Subjects with epidemiological exposure to SARS-CoV-2, or travel history to areas of moderate/high risk or abroad within the past 21 days;

15) Those who are judged by the investigator with any contradiction to the study protocol or the signing of informed consent being affected due to various medical, psychological, social, or other conditions.

7.4 Premature Termination Criteria

Premature termination refers to the situation where the subject fails to attend the end visit specified in the protocol, no further study procedures are required, no further follow-up is required, and no more information of the subject is collected since the last visit.

1) Subjects request to withdraw from the clinical trial;

2) Failing to complete the study process by leaving the observation area is deemed as voluntary withdrawal;

3) The subject's health status causes inappropriateness for study participation;

4) Any other reasons deemed by the investigator.

7.5 Rejection Criteria

The following criteria should be evaluated in each visit. Possibility of the subject being analyzed as the per protocol set (PPS) population will be compromised without study withdrawal if any of the following occurs:

1) Failing to meet the inclusion criteria;

2) Meeting the exclusion criteria;

3) No data or information collected in follow-ups after vaccination;

4) Excessive information and data missing after randomization;

5) Subjects who have not been vaccinated, received wrong immunization, or received an incorrect dose.

8 METHODS AND PROCEDURES

8.1 Selection of Subjects

The recruitment for the clinical trial will be performed at the study site approximately two weeks prior to visit 1 (V1). The number of subjects to be enrolled is about 1.5 times of the sample size (may be adjusted according to the actual situation). Investigators with relevant experience will perform such recruitment.

8.2 Informed Consent

The investigator should comply with relevant regulations, GCP, and the ethical principles specified in the "Declaration of Helsinki" during obtaining and recording the informed consent. Before the start of the study, the investigator should obtain the written approval/consent from the Ethics Committee on the informed consent form and other documents provided to the subjects.

Prior to participation in this clinical trial, the investigator should explain the informed consent form to the volunteers and their witnesses and provide sufficient time for them to inquire about the details of the trial before signing the informed consent form. While explaining to multiple persons, the investigator should allow each volunteer and/or witness to ask questions individually before they sign the informed consent form.

The investigator must retain the informed consent form signed by each volunteer for inspection by the pharmaceutical administration department or regulatory personnel. Each subject should be given a copy of the signed and dated informed consent form.

Informed consent must be obtained from each subject.

8.3 On-Site Registration

After the informed consent is obtained from and the informed consent form signed by a volunteer, the investigator will assign a screening number to the volunteer at the registry according to the arrival sequence. The screening number consists of S + site number + 4-digit serial number, starting from SXX0001 for each site.

8.4 Physical Examination and Screening

Subjects are required to undergo a general physical examination, including height/body weight/body temperature and blood pressure. Females of child-bearing age (before menopause) are required to undergo a urine pregnancy test. Subjects in the immunogenicity group are required to undergo immunogenicity, serum IgA, and salivary SIgA tests. Subjects 0001–0060 from a single site managed by the Jiangsu CDC will be included in the cellular immunity group and undergo cytokine testing.

The interviewing physician should conduct medical history inquiry and screening in accordance with the "Inclusion and Exclusion Criteria". Only subjects qualified by screening can be enrolled and randomized.

8.5 Subject Randomization

The investigator will assign each subject in the immunogenicity group a unique randomization number via the IWRS system according to the arrival sequence, without skipping or saving numbers.

8.6 Vaccine and Use

The subject information should be verified before vaccination. Only those who meet the protocol requirements can be vaccinated. Emergency medication and equipment such as epinephrine hydrochloride, simple ventilator, and ECG monitor should be available on-site.

8.6.1 Investigational vaccine

Ad5-nCoV-IH was jointly developed by the Beijing Institute of Biotechnology and CanSino Biologics Inc. The ICV vaccine was developed by Sinovac Biotech Ltd., Beijing Institute of Biological Products Co., Ltd., Wuhan Institute of Biological Products Co., Ltd., and Shenzhen Kangtai Biological Products Co., Ltd.

8.6.2 Immunization route and immunization procedure

Subjects will receive one dose of the investigational vaccine by intramuscular injection or nebulized inhalation at V1 according to the single-dose immunization procedure. The vaccine should be shaken well before use and used immediately after opening. The vaccine should not be used if it expires or has cracks, illegible label, or abnormal physical appearance. Subjects in the intramuscular injection group will receive the vaccine via intramuscular injection at the attachment site of lateral deltoid of the upper arm. The maximum volume of the syringe used for vaccination is recommended to be 1 mL, with a minimum scale of 0.01 mL. Subjects in the nebulized inhalation group will use a nebulizer for oral inhalation.

Nebulized inhalation procedures:

A special device for nebulized inhalation immunization is used.

(1) Connect the device to the nebulizer cup and ensure that the cup is upright.

(2) Withdraw corresponding volume of the vaccine with a syringe and inject the investigational vaccine into the area where the nebulization occurs.

(3) Start the nebulizer and atomize all the vaccine into the nebulizer cup. Remove the nebulizer after nebulization is completed.

(4) After taking a deep breath, the subject holds the mouthpiece of the nebulizer cup in the mouth, breathes slowly until there is no more mist, and then hold the breath for 5 seconds to complete the immunization.

Precautions:

(1) The investigational vaccine should be added to the bottom of the cup, without being splashed onto the walls of the cup. The needle should not come into contact with the bottom of the cup.

(2) The subject do not need to clean the mouth before vaccination but need to fast for food and water for half an hour after completing vaccination.

(3) The vaccination device can be reused, but the nebulizer cup can only be used once. The vaccination device should be handed over to designated personnel after the end of immunization on the same day.

(4) Nebulizer cups should be kept upright during atomization and horizontal during inhalation. Handle the nebulizer cups gently and do not shake or press them.

(5) The subject inhales this product through deep breathing, and after the inhalation is completed, it is required to hold the breath for 5 seconds before exhaling.

(6) One nebulizer cup can only atomize 0.1 mL.

8.6.3 Storage and transportation of investigational vaccines

1) Vaccine packaging: As this is an open-label study, blinded packaging is not required.

2) Vaccine storage: The investigational vaccines shall be stored in a safe and locked place, and shall not be accessed by any person unauthorized. The vaccines should be stored at 2–8 °C. The storage temperature should be recorded once every morning and afternoon on each working day.

3) Vaccine transportation: CanSino Biologics Inc. is responsible for transporting the Ad5- nCoV-IH vaccine from the manufacturing site to the clinical trial sites under refrigeration and submitting the investigational vaccines to the study sites along with the shipping temperature records (meeting the cold chain temperature for the vaccine) and the certificate of analysis (qualified), which should be checked, signed, and received by the vaccine manager of each clinical trial site. Each study site will provide ICV vaccines.

Each freezer, refrigerator, and cold chain equipment should be equipped with thermometers for investigational vaccine storage on the vaccination site and during transportation from the study site to the vaccination site and vice versa. The vaccine manager shall record the temperature every 30 min, including detailed transportation and storage temperature records. The vaccines must be transported within the storage temperature (2–8 °C). Any temperature deviations must be reported to the study center for further instructions. All vaccine shipments must be documented.

8.6.4 Concomitant medication

If a subject experiences a medical event during the study, appropriate treatment and medical treatment will be allowed, but the medication or medical treatment should be recorded in a timely manner. Any types of vaccines administered to the subjects during the study are required to be recorded in detail.

The concomitant medications of subjects within 28 days after each vaccination should be recorded in the diary card, and the concomitant medications for SAEs throughout the entire observation period should be entered in the EDC system.

Subjects are not recommended to receive other vaccines during the study, except for emergent vaccination due to emergencies involving rabies vaccine, tetanus vaccine, and other vaccines required. The concomitant vaccines used within 28 days after vaccination should be recorded in the diary card.

8.6.5 Organization and management of investigational vaccines

The investigator is responsible for the vaccination, recovery, and inventory checking of the investigational vaccines, as well as accurate and timely recording and archiving. At the end of the study, the investigator should return all remaining investigational vaccines and their packaging materials to the study center. The investigator may not use the investigational vaccine under any circumstance or on any occasion not specified in this protocol.

8.7 Safety Follow-Up and Evaluation

8.7.1 Safety observation

All subjects will remain on site for a 30-min observation period after receiving the vaccination. Investigators will observe each and every one of the subjects and record both regional and systemic responses reported during the 30-min observation period, as well as their severity.

A safety follow-up will be performed within 14 days after vaccination for all subjects, during which the investigator will systematically examine each subject for ARs, and the subject will fill in the "Vaccination Diary Card (Within 14 Days)" according to their symptoms and signs. From day 15 to day 28 after vaccination, post-vaccination AEs should be observed through spontaneous reporting and regular follow-ups. The investigators will inquire and check the diary cards at the next visit and guide the subjects in completing the cards. From day 28 to month 6 after vaccination, safety follow-ups on SAEs should be performed by spontaneous reporting.

8.7.2 Contents and parameters of safety observation

Based on the types and incidence of ARs observed in previous clinical trials as well as the ARs listed in the package inserts of similar products available on the market, this study has adhered to the "Guidelines for Grading Criteria of Adverse Reactions in Clinical Trials of Preventive Vaccines" (No. [2019]102 issued by NMPA). The contents and parameter grades of post-vaccination safety observations are presented in Tables 8.7-1 to 8.7-3, and the grading of vital sign-related safety is shown Table 8.7-4.

Table 8.7-1. Grading of administration-site (local) AEs of the nebulized inhalation vaccine

Symptoms/Signs	Grade 1	Grade 2	Grade 3	Grade 4
Dry mouth	Transient, not requiring treatment, and not affecting daily activities	Dry mouth, slightly affecting daily activities	Seriously affecting daily activities and requiring treatment	NA
Hoarseness	Transient, not requiring treatment, and not affecting daily activities	Hoarseness, affecting normal speech	Seriously affecting daily activities and requiring treatment	NA
Mucositis oral	Transient, not requiring treatment, and not affecting daily activities	Slightly affecting daily activities	Seriously affecting daily activities and requiring treatment	NA
Throat swelling	Transient, not requiring treatment, and not affecting daily activities	Throat redness and swelling, slightly affecting swallowing function	Seriously affecting daily activities and requiring treatment	NA
Pharyngalgia	Transient, not requiring treatment, and not affecting daily activities	Pain pharynx, slightly affecting daily activities	Severe pharyngalgia, seriously affecting daily activities, and requiring drug treatment	NA

Table 8.7-2. Grading of injection-site (local) AEs of the intramuscular vaccine

Symptoms/Signs	Grade 1	Grade 2	Grade 3	Grade 4
Pain	Not affecting or slightly affecting limb activities	Affecting limb activities	Affecting daily activities	Leading to loss of basic self-care ability or hospitalization
Induration*, swelling** #	Diameter of 2.5 cm to < 5 cm or area of 6.25 cm^2 to < 25 cm^2 and not or slightly affecting daily life	Diameter of 5 cm to < 10 cm or area of 25 cm^2 to < 100 cm^2 or affecting daily life	Diameter ≥ 10 cm, or area ≥ 100 cm^2, or ulceration, secondary infection, phlebitis, aseptic abscess, or wound drainage, or seriously affecting daily activities	Abscess, exfoliative dermatitis, or necrosis of dermal or deep tissues
Rash* and redness** #	Diameter of 2.5 cm to < 5 cm or area of 6.25 cm^2 to < 25 cm^2 and not or slightly affecting daily life	Diameter of 5 cm to < 10 cm or area of 25 cm^2 to < 100 cm^2 or affecting daily life	Diameter ≥ 10 cm, or area ≥ 100 cm^2, or ulceration, secondary infection, phlebitis, aseptic abscess, or wound drainage, or seriously affecting daily activities	Abscess, exfoliative dermatitis, or necrosis of dermal or deep tissues
Pruritus	Pruritus at the injection site, relieved spontaneously or within 48 h after treatment	Pruritus at the injection site, not relieved within 48 h after treatment	Affecting daily activities	NA
Cellulitis	NA	Requiring non-injection treatment (e.g., oral antibacterial, antifungal, and antiviral treatment)	Requiring intravenous injection treatment (e.g., intravenous antibacterial, antifungal, and antiviral treatment)	Sepsis or tissue necrosis, etc.

Notes: * In addition to grading and evaluation by direct measurement of diameter, progress and changes in the measurement results should also be recorded.

** The greatest measured diameter or area should be used.

For the evaluation and grading of induration and swelling, rash, and redness, indicators with greater grades should be selected based on the functional grades and actual measurement results.

Table 8.7-3. Grading of non-injection-site (systemic) AEs (sore throat is a systemic AE in the case of intramuscular injection)

Symptoms/Signs of organs/systems	Grade 1	Grade 2	Grade 3	Grade 4
Diarrhea	Mild or transient, 3–4 times/day, abnormal stool properties, or mild diarrhea for less than 1 week	Moderate or persistent, 5–7 times/day, abnormal stool properties, or diarrhea > 1 week	> 7 times/day, abnormal stool properties, or hemorrhagic diarrhea, orthostatic hypotension, electrolyte imbalance, and requiring intravenous infusion > 2 L	Hypotensive shock, requiring hospitalization
Nausea	Transient (< 24 h) or intermittent, normal food intake	Persistent nausea resulting in decreased food intake (24–48 h)	Persistent nausea resulting in minimal food intake (> 48 h) or rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Anorexia	Decreased appetite, without reduced food intake	Decreased appetite associated with decreased food intake, without significant weight loss	Decreased appetite, associated with significant weight loss	Intervention indicated (e.g., gastric tube feeding and parenteral nutrition)
Vomiting	1–2 episodes/24 h and no interference with activity	3–5 episodes/24 h or some interference with activity	Over 6 episodes within 24 h or requiring intravenous fluids	Requiring hospitalization or other nutritional supplement ways for hypotensive shock
Fatigue and asthenia	Not affecting daily activities	Affecting normal daily activities	Seriously affecting daily activities, unable to work	Requiring emergency treatment or hospitalization
Headache	Not affecting daily activities, not requiring treatment	Transient, slightly affecting daily activities, possibly requiring treatment or intervention	Seriously affecting daily activities, requiring treatment or intervention	Refractory, requiring emergency treatment or hospitalization
Arthralgia	Mild pain, not interfering with functions	Moderate pain, requiring analgesics and/or pain hinders the function, but not affecting daily activities	Severe pain, requiring analgesics and/or pain affecting daily activities	Disabling pain

Symptoms/Signs of organs/systems	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Chest pain	Mild pain, not interfering with functions	Moderate pain, requiring analgesics and/or pain hinders the function, but not affecting daily activities	Severe pain, requiring analgesics and/or pain affecting daily activities	Disabling pain
Pharyngalgia	Transient, not requiring treatment, and not affecting daily activities	Pain pharynx, slightly affecting daily activities	Severe pharyngalgia, seriously affecting daily activities, and requiring drug treatment	NA
Rhinorrhea	Transient, not requiring treatment	Persistent, treatment effective	Treatment uncontrollable	Requiring emergency treatment or hospitalization
Sneezing	Transient, not requiring treatment	Persistent, treatment effective	Treatment uncontrollable	Requiring emergency treatment or hospitalization
Cough	Transient, not requiring treatment	Persistent cough, treatment effective	Paroxysmal cough, treatment uncontrollable	Requiring emergency treatment or hospitalization
Muscle pain at non-injection sites	Not affecting daily activities	Slightly affecting daily activities	Severe muscle pain, seriously affecting daily activities	Requiring emergency treatment or hospitalization
Pruritus at non-injection sites (no skin damage)	Slight itching, not affecting or slightly affecting daily activities	Pruritus affecting daily activities	Pruritus, causing inability to perform daily activities	NA
Skin and mucosal abnormalities	Erythema/pruritus /color changes	Diffuse skin rash/rash maculo-papular/dryness/desquamation	Blister/exudation/desquamation/ulceration	Dermatitis exfoliative involving mucosa, erythema multiforme, or suspected Stevens-Johnsons syndrome
Acute anaphylactic reactions**	Local urticaria (blister), not requiring treatment	Local urticaria requiring treatment, or mild angioedema not requiring treatment	Extensive urticaria or angioedema requiring treatment, or mild bronchospasm	Anaphylactic shock, or life- threatening bronchospasm, or laryngeal edema

Symptoms/Signs of organs/systems	Grade 1	Grade 2	Grade 3	Grade 4
Syncope	Near syncope, without loss of consciousness (e.g., presyncope)	Loss of consciousness, not requiring treatment	Loss of consciousness, requiring treatment or hospitalization	NA
Constipation*	Requiring stool softeners and diet adjustment	Requiring laxatives	Obstipation, requiring manual bowel movement or enema	Toxic megacolon or bowel obstruction
Dysphagia	Mild discomfort when swallowing	Restricted diet	Great limitations in eating and talking; unable to consume solid food	Unable to consume liquid food; requiring intravenous nutrition
Arthritis	Mild pain, with inflammation, erythema, or joint swelling; but not interfering with functions	Moderate pain, with inflammation, erythema, or swelling of the joints; interfering with functions, but not affecting daily activities	Severe pain, with inflammation, erythema, or swelling of the joints; affecting daily activities	Permanent and/or disabling joint injuries
New-onset convulsion	NA	NA	1–3 convulsion(s)	Prolonged and multiple convulsions (e.g., status epilepticus) or uncontrollable convulsions (e.g., refractory epilepsy)
Acute bronchospasm	Transient, not requiring treatment; FEV_1%: 70%–80%	Requiring treatment: return to normal after treatment with bronchodilators; FEV_1%: 50%–70%	Unable to return to normal after treatment with bronchodilators; FEV_1%: 25%–50%, or persistent intercostal retraction	Cyanosis, FEV_1%: < 25%, or requiring intubation
Dyspnea	Dyspnea during exercise	Dyspnea during normal activities	Dyspnea at rest	Dyspnea, requiring oxygen therapy, hospitalization, or assisted breathing
Insomnia*	Slight sleep problem, not affecting or slightly affecting daily activities	Moderate sleep problem, affecting daily activities	Severe sleep problem, seriously affecting daily activities and requiring treatment or hospitalization	NA
Irritation or suppression	Mild irritation or mild suppression	Irritability or lethargy	Inability to be soothed or poor response	NA

Symptoms/Signs of organs/systems	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Mental disorders (including anxiety, depression, mania, and confusion), for which the detailed symptoms should be reported	Mild symptoms, not requiring medical consultation, or the act not affecting or slightly affecting daily activities	Presence of clinical symptoms, requiring medical consultation, or the act affecting daily activities	Requiring hospitalization, or behavioral ability not supporting the daily life	Tending to harm oneself or other people, or acute mental confusion, or loss of ability for basic self- care
Pain at non-injection sites# (sites should be identified when reporting)	Slight pain, not affecting or slightly affecting daily activities	Pain, affecting daily activities	Pain, causing inability to perform daily activities	Disabling pain, leading to loss of basic self- care ability
Fever※ [axillary temperature (°C)]	37.3 to < 38.0	38.0 to < 38.5	38.5 to < 39.5	≥ 39.5, lasting ≥ 3 days

Notes: FEV_1% = Forced expiratory volume in one second (FEV_1)/Forced vital capacity (FVC)

* Changes in constipation and insomnia before and after vaccination should be noted.

** Type I hypersensitivity.

\# Pain at non-injection sites other than muscle pain, arthralgia, and headache.

※ Generally, the axillary temperature is used in China and converted to the oral temperature and rectal temperature as necessary. Generally, oral temperature = Axillary temperature + 0.2 °C; rectal temperature = axillary temperature + (0.3–0.5 °C). In the case of persistent hyperpyrexia, its cause should be identified where possible.

Local AEs are all solicited events. Among the above non-injection site (systemic) AEs, solicited AEs include sore throat (considered a systemic AE in the case of intramuscular injection), fever, diarrhea, fatigue, asthenia, nausea, anorexia, vomiting, headache, cough, arthralgia, chest pain, muscle pain at non-injection sites, pruritus at non-injection sites (without skin damage), skin and mucosal abnormalities, rhinorrhea, and sneezing, while the rest are unsolicited AEs. Refer to the Investigator's Brochure for other non-solicited AEs.

General rules for grading of other AEs:

For clinical abnormalities not described in the grading tables above, the severity grading and assessment for AEs are conducted according to the following criteria:

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Mild: short-term (< 48 h) or mild discomfort, not affecting activities and not requiring treatment	Moderate: mild or moderate restricted activities, potentially requiring medical consultation, and not requiring treatment or only requiring mild treatment	Severe: obviously restricted activities, requiring medical consultation and treatment, and potentially requiring hospitalization	Critical: potentially life-threatening, severely restricted activities, and requiring monitoring and treatment	Death

8.7.3 Outcomes of adverse events

The outcome of AEs include: 1) resolved; 2) resolved with sequelae; 3) improved; 4) ongoing; 5) death; 6) lost to follow-up.

8.7.4 Relationship between adverse events and investigational vaccines

The investigators should try their utmost to explain the AEs and assess its possible causality, i.e., the causality between the investigational vaccination and the alternative causes (e.g., history of underlying diseases and concomitant treatments). This is applicable to all AEs, including serious AEs and non-serious AEs.

Causality assessment will be conducted according to the reasonableness of the explanation of events from one or more perspectives as follows:

- Response of similar nature observed previously in the drug products of the same kind;
- Same event reported in the literature on the drug products of similar kinds;
- Event temporally associated with vaccination of the investigational vaccine and shown again after its re-vaccination.

By definition, all solicited AEs (e.g., all solicitedly reported local AEs) at the injection sites are deemed related to vaccination. The causality between an AE and vaccination should be assessed by investigators according to their judgment on the presence/absence of a reasonable possibility of an AE occurring due to the vaccination:

- Related: A connection is suspected between the investigational vaccine and the AE (the extent of possibility cannot be determined); there is a reasonable possibility that the investigational vaccine leads to the AE.

- Not related: No relationship between the investigational vaccine and the AE is suspected; there are other more possible causes and the investigational vaccination is not suspected to contribute to the AE.

8.7.5 Handling of adverse reactions/events

AE: an unexpected medical event a subject experiences during the clinical trial, which is not necessarily caused by or related to the vaccine/vaccination.

AR: an unexpected or untoward reaction during the vaccination at the prescribed dose and according to prescribed procedures, which is usually related to vaccination.

Serious adverse event (SAE): an important medical event not necessarily caused by or related to clinical trials of vaccines, which leads to: 1) death; 2) life-threatening situation; 3) hospitalization or prolonged hospitalization; 4) permanent or significant disability/incapacity; 5) congenital anomaly or birth defect; or 6) other important medical events that may cause the conditions above without treatment.

Suspected unexpected serious adverse reaction (SUSAR): A suspected AR refers to an untoward and unintended reaction at any dose, analyzed to be at least possibly related to the drug; an unexpected AR refers to that the nature, severity, outcome, or frequency of the AR is inconsistent with the expected risks described in the protocol or other relevant information (e.g., the Investigator's Brochure and package insert).

Subjects should report any clinically significant diseases/events after vaccination to the investigator in a timely manner. The investigator should follow up the AR/AE until the symptoms disappear or become stable. Necessary treatment and management will be provided unconditionally when deemed necessary by the investigator in order to relieve the subject from suffering from the AR/AE. All medications and medical management will be recorded during each follow-up.

If a SAE/SAR occurs, the investigator should take necessary measures immediately, complete the "Serious Adverse Event Report Form", and report it to the PI and the study center via fax or email within 24 h.

8.7.6 Reporting procedures for serious adverse events

In case of any SAE, whether or not related to the investigational vaccines, the investigator must complete the "Serious Adverse Event Reporting Form" after being informed and report it to the PI and the vaccine manufacturer via fax or email within 24 h. The report should include a description, onset, type, duration, causality with vaccination, outcome, and treatment (symptomatic treatment) of the AE.

Upon receipt of a SAE report, the study center should confer with the investigator to determine if the subject should continue or discontinue trial participation in consideration of the duration, range, intensity, and outcome of the AE, as well as the subject's will. The study center should determine if the SAE is a SUSAR; if so, the study center should report it to the Center for Drug Evaluation, NMPA and the PI, and the PI should report the SUSAR to the Institutional Review Board.

For SUSARs which are fatal or life-threatening, the study center should, after the first knowledge, report them to the Center for Drug Evaluation, NMPA as soon as possible (not more than 7 natural days), report within the following 8 days and complete the follow-up information.

For SUSARs which are not fatal or life-threatening or other potentially serious safety risk information, the study center should, after the first knowledge, report to the Center for Drug Evaluation, NMPA (not more than 15 natural days). The investigator should record SAEs truthfully and evaluate and discuss them in the final report after trial completion or termination.

Table 8.7-4. Contact information of the Institutional Review Board

Institutional Review Board of Jiangsu Provincial Center for Disease Control and Prevention	Contact: Ba Lu Tel.: 025-83759406	E-mail: ec@jscdc.cn

8.7.7 Handling of pregnancy events

Subjects are not allowed to participate in the investigational vaccine immunization during pregnancy. Urine pregnancy tests will be performed on subjects before vaccination and those tested positive will not be enrolled. If a pregnancy event is collected within 6 months of follow-up visits, a "Pregnancy Event Reporting Form" should be completed.

8.8 Immunogenicity Evaluation

8.8.1 Sample collection

For the immunogenicity group, 10.0 mL of venous blood will be collected on day 0 (V1), day 14 (V2, time window of ± 3 days), day 28 (V3, time window of ± 5 days), month 3 (V4, time window of ± 15 days), and month 6 (V5, time window of ± 15 days) after vaccination, and 2.0 mL of saliva will be collected on day 0 (V1), day 14 (V2), day 28 (V3, time window of ± 5 days), month 3 (V4, time window of ± 15 days), and month 6 (V5, time window of ± 15 days) after vaccination. For subjects in the cellular immunity group, 15.0 mL of venous blood will be collected on day 0 (V1) and day 14 (V2, time window of ± 3 days) after vaccination.

Vacuum blood collection tubes containing serum separator gels are used. Sample refrigeration prior to centrifugation is not allowed in order to minimize the risk of hemolysis and to avoid blood cell contamination during serum transfer to the target serum tube. Serum is separated on the blood collection day and stored at −20 °C or below for serum antibody testing and backup.

According to the operating manual, the saliva samples will be stored at −20 °C or below and delivered to the testing laboratory in time.

Cellular immunity samples will be collected in vacuum blood collection tubes with anticoagulant. PBMC will be separated and used for ICS on the day of blood collection.

The serum and saliva samples collected in this clinical trial will be used to evaluate the immune response of the investigational vaccine. Approval by the Institutional Review Board and consent of the subjects are required if used for other research purposes. Except for serum and saliva samples that are sent for testing, remaining backup samples will be transferred to a third-party laboratory at the end of the clinical trial. Blood and saliva samples may not be delivered abroad.

8.8.2 Assay of anti-SARS-CoV-2 specific neutralizing antibodies

The serum anti-SARS-CoV-2 S neutralizing antibody levels will be tested in subjects in the immunogenicity group before booster immunization as well as 14 days, 28 days, 3 months, and 6 months after immunization to compare the differences in antibody levels and persistence of the two vaccines.

8.8.3 ELISA assay of anti-SARS-CoV-2 RBD protein IgG antibodies

The serum anti-SARS-CoV-2 RBD protein IgG antibody levels will be tested in subjects in the immunogenicity group before booster immunization as well as 14 days, 28 days, 3 months, and 6 months after immunization to compare the differences in antibody levels and persistence of the two vaccines.

8.8.4 Assay of anti-recombinant replication-defective human adenovirus type 5 neutralizing antibodies

The serum anti-recombinant replication-defective human adenovirus type 5 neutralizing antibody levels will be tested in subjects in the immunogenicity group before booster immunization to determine the effects of baseline anti-Ad5 neutralizing antibody levels on anti-SARS-CoV-2 antibody levels.

8.8.5 Assay of salivary SIgA

The SIgA antibody levels will be tested in subjects in the immunogenicity group before booster immunization as well as 14 days, 28 days, 3 months, and 6 months after immunization to compare the difference in SIgA antibody levels between the two vaccines.

8.8.6 Assay of serum IgA

The serum IgA antibody levels will be tested in subjects in the immunogenicity group before booster immunization as well as 14 days, 28 days, 3 months, and 6 months after immunization to compare the difference in IgA antibody levels between the two vaccines.

8.8.7 VOC/VOI cross-neutralization testing

The levels of cross-neutralizing antibodies to VOC/COI will be tested in subjects in the immunogenicity group before and 28 days after booster immunization to compare the protective effects of the two vaccines on VOC and VOI.

8.8.8 Assay of antigen-specific $CD4^+$ T cell and $CD8^+$ T cell responses

The seropositive rates and response levels of IFN-γ, TNF-α, IL-2, IL-4, IL-5, and IL-13 in PBMCs will be tested in the subjected in the cellular immunity group before and 14 days after booster immunization to compare the difference in antibody levels between the two routes of administration.

Calculation of uncertain values: For antibodies determined by ELISA and neutralization assay, their GMT, GMI, and seroconversion rate will be calculated based on half of the respective starting value when a negative result is observed at the starting dilution and based on the maximum dilution when the maximum dilution is exceeded.

8.8.9 Serum numbering

1) Test tube numbering for anti-SARS-CoV-2 neutralizing antibodies: Study ID-visit number-1
2) Test tube numbering for anti-SARS-CoV-2 RBD protein IgG antibodies (ELISA) and serum IgA: Study ID-visit number-2
3) Test tube numbering for anti-recombinant replication-defective human type 5 adenovirus neutralizing antibodies: Study ID-visit number-3
4) Test tube numbering for saliva samples: Study ID-visit number
5) Test tube numbering for antigen-specific $CD4^+$ T cell and $CD8^+$ T cell responses: Study ID- visit number-P
6) Test tube numbering for backup serum samples: Study ID-visit number-3 (except blood before immunization) and study number-visit number-4.

8.8.10 Storage and transportation of samples

Storage and transportation of samples will be performed using unified operating standards. The samples should be stored at −20 °C or below and delivered to the testing laboratory in time.

8.9 On-Site Workflow

8.9.1 Overview of visit procedures

From the beginning of the screening prior to the enrollment to the end of the study, the subjects will complete all the study content in 5 or 6 visits. The specific time, time window, and content for each visit are detailed in 8.9-1.

Table 8.9-1. Visit procedures

Visit point	V1	V2	V3	V4	V5
Visit time	D-7-D0	D14	D28	Month 3	Month 6
Time window	/	± 3 days	± 5 days	± 15 days	± 15 days
Informed consent	●				
Registration/identity verification	●	●	●	●	●
Physical examination (blood pressure, height, weight, body temperature)	●				
Urine pregnancy (female of childbearing potential)	●				
Medical history inquiry and eligibility screening	●				
Randomization number assignment[a]	●				
Immunogenicity blood sampling[a]	10 mL	10 mL	10 mL	10 mL	10 mL
Cellular immunity blood sampling[b]	15 mL	15 mL			
Saliva sampling[c]	2 mL	2 mL	2 mL	2 mL	2 mL
Immunization[d]	●				
Dispensation of diary card (0–14 days)	●				
Return of diary card (0–14 days) Dispensation of diary card (after 14 days)		●			
Return of diary card (after 14 days)			●		
Safety observations[e]	●	●	●		
SAE observation and reporting[f]	●	●	●	●	●

a. Immunogenicity group only;

b. Subjects in the immunogenicity group recruited from Jiangsu CDC who are included in cellular immunity group only;

c. Immunogenicity group only;

d. If the investigator believes the health status of the subject on the day of vaccination is temporarily unsuitable for vaccination, the dose may be delayed up to 1 week;

e. All adverse events within 0–28 days after immunization should be observed and documented, regardless of whether related to the vaccine;

f. The investigator should fill out the "Serious Adverse Event Report Form" and complete the initial report within 24 h of learning of an SAE and complete the final report at the end of the event. Except for subjects in the immunogenicity subgroup, all subjects should complete V4–V5 via telephone

Table 8.9-2. Sample collection schedule at clinical trial visits

Visit	V1	V2	V3	V4	V5
Visit time	D0	D14	D28	Month 3	Month 6
Immunogenicity[#]	10 mL	10 mL	10 mL	10 mL	10 mL
Saliva[#]	2 mL	2 mL	2 mL	2 mL	2 mL
Cellular immunity[@]	15 mL	15 mL			

Note: # Immunogenicity group only

@ Subjects in the Jiangsu CDC who are included in cellular immunity group only

8.9.2 Grouping and assignment of on-site personnel

Table 8.9-3. Grouping and assignment of on-site personnel

Serial no.	Group	Responsibility	Visit
1)	Informed consent	Obtain informed consent from subjects	V1
2)	Registration	Perform registration and identity verification	V1–V5
3)	Physical examination	Perform general physical examination prior to enrollment	V1
4)	Urine test	Collect urine for urine pregnancy test	V1
5)	Screening	Conduct inquiry in accordance with the inclusion and exclusion criteria and complete screening forms	V1
6)	Study number assignment	Assign randomization numbers	V1
8)	Immunogenicity blood sampling	Collect 10.0 mL of venous blood	V1–V5
9)	Saliva sampling	Collect 2.0 mL of saliva	V1–V5
10)	Cellular immunity blood sampling	Collect 15.0 mL of venous blood	V1, V2
9)	Vaccination	Perform vaccination according to the procedure and route	V1
10)	On-site observation	Observe subjects for at least 30 min after vaccination	V1
11)	Diary card collection	Collect diary cards and review the format and accuracy of completed diary cards	V2, V3

8.9.3 Site management

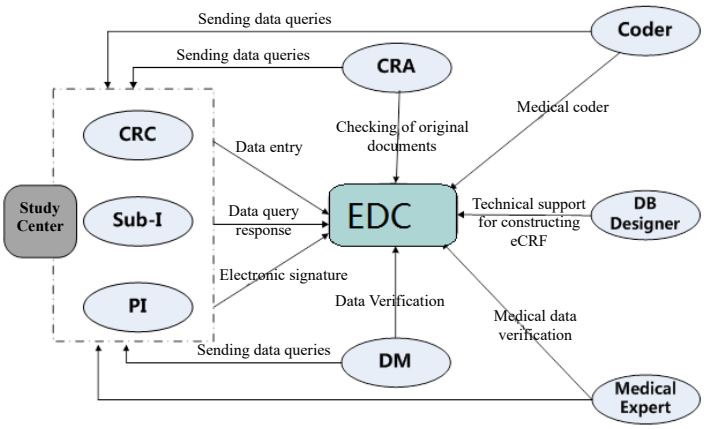
Table 8.9-4. Grouping and assignment for site management

Serial no.	Group	Responsibility	Visit
1)	Medical guide	1) Maintain on-site order; 2) If necessary, lead subject to each testing group to complete all tests.	V1–V5
2)	Serum separation and management	1) Receive blood samples delivered to the site and fill in the handover form; 2) Isolate serum and aliquot into EP tubes; 3) Store and regularly transport biological specimens; 4) Record the type, quantity, and ID numbers of transported specimens.	V1–V5
3)	Saliva management	1) Receive saliva samples delivered to the site and fill in the handover form; 2) Saliva sample treatment for preservation; 3) Store and regularly transport saliva samples; 4) Record the type, quantity, and ID numbers of transported specimens.	V1-V5
4)	Vaccine management	1) Receive and store vaccines; 2) Record the cold chain temperature; 3) Fill in vaccine issuance and recovery records; 4) Reconcile vaccine quantities at time of locking.	V1
5)	Safety observation	1) Routine safety follow-up; 2) Investigate serious adverse events; 3) Report serious adverse events in a timely manner;	V1–V5
6)	Document management	1) Collect and manage information onsite; 2) Process records; 3) Archive files.	V1–V5
7)	Logistic support	1) Ensure materials meeting requirements; 2) Ensure vehicles meeting requirements.	V1–V5
8)	Emergency care	1) Handle safety incidents and emergencies which require professional ambulance personnel to be onsite; 2) In the event of serious cases, send to the hospital immediately.	V1
9)	Quality control	1) Perform check prior to study initiation; 2) Perform check in one week after subject enrollment; 3) Perform periodic checks during the study; 4) Perform checks at time of site-closing.	V1–V5

8.10 Data Management

In this study, Electronic Data Capture (EDC) system is used for the collection and management of study data, and the complete records of modification are retained by the system to ensure the traceability of the clinical trial data; data collection, entry, cleaning, consistency verification, database lock, and other tasks are completed in accordance with the Technical Guidelines for Data Management in Clinical Trials. The data management process should meet GCP regulations to ensure the authenticity, integrity, and accuracy of the clinical trial data. The main process of data management is shown below. See Data Management Plan (DMP) for additional details.

8.10.1 Data management work flow chart



8.10.2 Roles and responsibilities in data collection

Role	Abbreviations	Responsibility
Clinical data associate	CRC	1. Enter data; 2. Answer queries;
Assistant investigator	Sub-I	1. Enter data; 2. Answer queries;
Principal Investigator	PI	1. Enter data; 2. Answer queries; 3. Approve for confirmation;
Clinical research associate	CRA	1. Verify the consistency of source files 2. Issue queries; 3. Close queries;
Project manager	PM	1. Read only;
Data management	DM	1. Issue queries; 2. Close queries; 3. Freeze/thaw data; 4. Lock data;
Medical coder	Coder	1. Encode; 2. Issue queries.

8.10.3 Design and establishment of database

The database designer establishes the project database (eCRF) in accordance with the CDISC criteria as far as possible.

After the database is established and tested, the authorized persons of the roles, e.g., PI, Sub-I, CRC, PM, CRA, and DM, are trained prior to the formal online application.

The data manager writes the Data Management Plan (DMP), which shall be finalized before the screening of the first subject.

8.10.4 Data entry

The investigators or personnel authorized by the investigators will complete the data entry on-line in a timely manner after the visit.

The investigator must approve for confirmation of the data in eCRF, to demonstrate that the data recorded in the eCRF are true. After completion of data entry, any change to the data must be indicated with comments and will be recorded automatically in the system.

8.10.5 Monitoring of data records

The CRA shall conduct monitoring of data records entered into the EDC on a regular or irregular basis so as to ensure that all recorded data are consistent with the original documents. In the event of inconsistencies, the CRA should send the queries to the investigators in the corresponding section of the EDC system. The investigators should verify the raw data and update the recorded contents until all inconsistencies are resolved in the EDC system. Before locking the database, the monitor should verify the raw data of the subjects carefully and the necessary signatures of the investigators.

8.10.6 Data verification

The data manager conducts query management for the trial data in accordance with the data verification plan (DVP).

When the data is entered into the EDC system, the system automatically verifies any illogical data and issues a query; such a query will be reviewed and answered by the investigator or an authorized person. When updated data renders the logical verification invalid, the query will be closed automatically; DM can review an automatically closed query. If the problem is not solved, then DM can manually add a query and communicate with the study site until the problem is solved.

In addition to automatic verification by the system, the queries can be manually added in the EDC system when the investigator is required to clarify/verify/confirm queries found via SAS programming or manual verification by the data manager.

Before locking the database, the data manager shall ensure that all queries have been cleaned up, and that the investigator has completed the electronic signatures in the EDC system. This is to ensure the integrity and accuracy of the data of patients.

8.10.7 Medical coding

Medical coders conduct medical coding. Medical coding is performed on unsolicited AEs. AEs are encoded in accordance with MedDRA (version 21.1 or higher).

During coding, DM can send queries to the investigators on-line in a real-time manner for coding failure due to improper, inaccurate, or vague medical terminology.

Medical review of medical coding is required before locking the database.

8.10.8 Database locking

The database locking list shall be completed. According to the procedures for database locking, written approval for database locking shall be signed by the data manager, data analysts, representatives of clinical research associates, representatives of investigators, etc., and the data manager will export it to the database in a specified format, and deliver it to data analysts for statistical analysis. After locking the database, if there is definite evidence warranting unlocking, the investigators and related personnel shall sign the unlocking document.

8.10.9 External data management

The immunogenicity data is managed as external data. The requirements on data transmission are shown in the "External Data Transmission Protocol". For data management, external data is subjected to review and consistency verification.

8.10.10 eCRF archiving

After the end of the trial, the eCRF of each patient is exported to PDF for electronic archiving, and is saved into a compact disc and stored in the clinical trial institution.

8.11 Statistical Analysis

8.11.1 Statistical analysis plan

A statistics team (third-party) should be entrusted to carry out statistical analysis and participate in the whole process, from study design, study implementation to analysis and summary. After the trial protocol has been completed and approved by the Ethics Committee, the investigator and statistics team should coordinate the establishment of the database and formulation of statistical analysis plan, before submitting them to the principal investigator, biostatistician, and data manager for review and approval, in order to determine the following: selection of analysis dataset, statistical methods, etc.

8.11.2 Study hypotheses

This study will test a superiority hypothesis. The immunogenicity of 1 booster dose of Ad5 (adenovirus type 5 vector) vaccine for inhalation is non-inferior to that of 1 intramuscular injection of inactivated COVID-19 vaccine.

The superiority test will be carried out with the following hypothesis:

$$H_0 \text{ (null hypothesis): } T/C \leq 1$$

$$H_1 \text{ (alternative hypothesis): } T/C > 1$$

Superiority will be established when the lower limit of the one-sided 97.5% confidence interval of the GMT ratio ($GMT_{1 \text{ dose of inhalation}}/GMT_{1 \text{ dose of ICV}}$) of anti-SARS-CoV-2 neutralizing antibodies is greater than 1.

Testing strategy: When superiority is established in the overall population, superiority will also be tested in different age groups (18-59 years old, ≥ 60 years old). Age-stratified results will be used as supportive data. No adjustments will be made to the α.

8.11.3 Selection of analysis sets

Safety set (SS): All randomized subjects who receive the immunization will be included in the safety evaluation. Data in violation of the protocol were not rejected.

Immunogenicity evaluation datasets:

Full analysis set (FAS): FAS included an ideal subject population determined by the intention-to-treat (ITT) principles. All subjects enrolled and vaccinated were included in the FAS, whether or not they had positive baseline antibody levels or major deviation from the protocol.

Per protocol set (PPS): PPS is a subset of FAS; the subjects in this dataset are more compliant with the protocol, have no major protocol violations during the study, and meet all eligibility criteria.

PPS will be used as the primary analysis dataset for immunogenicity analysis in this trial. However, FAS will be analyzed as well. Any inconsistencies between the FAS and PPS results will be discussed in the report.

8.11.4 Data statistical method

Unless otherwise specified, the two-sided testing with $\alpha = 0.05$ and the two-sided 95% confidence interval are used for all statistical tests. Continuous variables are summarized with descriptive statistics, including number of subjects, mean, median, standard deviation, maximum, and minimum. Categorical parameters are described with the number and percentage of subjects in each category.

The statistical analysis will be further described in the Statistical Analysis Plan (SAP) which will be finalized before database locking.

8.11.4.1 Demographics and baseline characteristics

Demographics and baseline characteristics of each group and in total are summarized with descriptive statistics.

Demographics include: enrollment and visit completion (screening failure, protocol violation); the number of subjects from each group in each dataset, and the distribution of age and gender.

Baseline characteristics include: antibody distribution of each immunogenicity evaluation before immunization by group and in total.

8.11.4.2 Safety analysis

AEs are encoded using MedDRA. The analysis of AEs will be based on the treatment-emergent adverse events (TEAEs) after vaccination. A TEAE is defined as an AE that occurs during vaccination or worsens during vaccination compared with the state before vaccination. The incidence of AEs will be summarized by System Organ Class (SOC) and Preferred Term (PT). SAEs and AEs leading to study discontinuation will be similarly summarized and tabulated.

8.11.4.2.1 Incidence of adverse reactions within 30 min post-vaccination

The number of subjects, number of events, and percentage of adverse reactions occurring within 30 min post-vaccination in each group will be summarized. The number of subjects, number of events, and percentages of adverse reactions/events occurring within 30 min post-vaccination in each group by severity and symptoms will be summarized. The inter-group comparison is performed using chi-square test or Fisher's exact test.

8.11.4.2.2 Incidence of adverse reactions within 0–14 days post-vaccination

The number of subjects, number of events, and percentage of adverse reactions occurring within 0–14 days post-vaccination in each group will be summarized. The number of subjects, number of events, and percentage of adverse reactions occurring within 0–14 days post-vaccination in each group will be summarized by severity and symptom. The inter-group comparison is performed using chi-square test or Fisher's exact test.

8.11.4.2.3 Incidence of adverse events/adverse reactions within 0–28 days post-vaccination

The number of subjects, number of events, and percentage of adverse reactions/adverse events occurring within 0–28 days post-vaccination in each group will be summarized. The number of subjects, number of events, and percentage of adverse reactions/adverse events occurring within 0–28 days post-vaccination in each group will be summarized by severity and symptom. The inter- group comparison is performed using chi-square test or Fisher's exact test.

8.11.4.2.4 SAEs within 6 months post-vaccination

The number of subjects, number of events, and percentage of SAEs occurring within 6 months post-vaccination in each group will be summarized. The number of subjects, number of events, and percentage of SAEs occurring within 6 months post-vaccination in each group will be summarized by severity and symptom. The inter-group comparison is performed using chi-square test or Fisher's exact test.

8.11.4.3 Immunogenicity analysis

8.11.4.3.1 Geometric mean titer (GMT) of anti-SARS-CoV-2 specific neutralizing antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after booster immunization

The GMT in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartile, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The reverse cumulative distribution is plotted. The analysis of GMC of anti-SARS-CoV-2 S-RBD IgG antibodies will be stratified by baseline anti- Ad5 neutralizing antibody titer and age.

The GMI in each group will be summarized using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The GMI of anti-SARS-CoV-2 S-RBD IgG antibodies will be compared between the two routes of administration, stratified by baseline anti-Ad5 neutralizing antibody titer and age.

The seroconversion rate of each group is summarized and indicated using number of subjects and percentages to calculate the 95% CI of seroconversion rate. Inter-group differences will be compared using chi-square test or Fisher's exact test. The seroconversion rates of anti-SARS-CoV-2 S-RBD IgG antibodies between the two routes of administration will be compared, stratified by baseline anti-Ad5 neutralizing antibody titer.

8.11.4.3.2 GMC, GMI, and seroconversion rate of anti-SARS-CoV-2 RBD protein IgG antibodies in subjects in the immunogenicity group at 14 days, 28 days, 3 months, and 6 months after immunization;

The GMC in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartile, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The reverse cumulative distribution is plotted. The analysis of GMC of anti-SARS-CoV-2 S-RBD IgG antibodies will be stratified by baseline anti- Ad5 neutralizing antibody titer and age.

The GMI in each group will be summarized using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The GMI of anti-SARS-CoV-2 S-RBD IgG antibodies between the two routes of administration will be compared, stratified by baseline anti-Ad5 neutralizing antibody titer and age.

The seroconversion rate of each group is summarized and indicated using number of subjects and percentages to calculate the 95% CI of seroconversion rate. Inter-group differences will be compared using chi-square test or Fisher's exact test. The seroconversion rate of anti-SARS-CoV-2 S-RBD IgG antibodies between the two routes of administration will be compared, stratified by baseline anti-Ad5 neutralizing antibody titer.

8.11.4.3.3 GMT, seroconversion rate, and GMI of certain VOC/VOI cross-neutralizing antibodies in subjects in the immunogenicity group at 28 days after booster immunization

The geometric mean titer (GMT) of each group at each visit are summarized and described using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum values, etc. Inter-group differences will be compared using a t test. The reverse cumulative distribution is plotted. The analysis of GMT of VOC/VOI cross-neutralizing antibodies will be stratified by baseline anti-Ad5 neutralizing antibody titer and age.

The seroconversion rate of each group is summarized and indicated using number of subjects and percentages to calculate the 95% CI of seroconversion rate. Inter-group differences will be compared using chi-square test or Fisher's exact test. The GMI in each group will be summarized using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test.

8.11.4.3.4 GMC, seroconversion rate, and GMI of salivary sIgA antibodies in subjects in the immunogenicity group at 14 days, 3 months, and 6 months after immunization

The GMC in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartile, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The reverse cumulative distribution is plotted.

The GMI in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test.

The seroconversion rate of each group in each visit is summarized and indicated using number of subjects and percentages to calculate the 95% CI of seroconversion rate. Inter- group differences will be compared using chi-square test or Fisher's exact test.

8.11.4.3.5 GMC, seroconversion rate, and GMI of serum IgA antibodies in subjects in the immunogenicity group at 14 days, 3 months, and 6 months after immunization

The GMC in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartile, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test. The reverse cumulative distribution is plotted.

The GMI in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartiles, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test.

The seroconversion rate of each group in each visit is summarized and indicated using number of subjects and percentages to calculate the 95% CI of seroconversion rate. Inter- group differences will be compared using chi-square test or Fisher's exact test.

Stratified by baseline anti-Ad5 neutralizing antibody titer and age.

8.11.4.3.6 Seropositive rate and response levels of IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL- 13 in $CD4^+$ and $CD8^+$ T cells in subjects in the immunogenicity group at 14 days after immunization

The response levels in each group at each visit will be summarized using number of subjects, geometric mean, standard deviation, median, quartile, maximum, minimum, and 95% CI, etc. Inter-group differences will be compared using a t test.

The seroconversion rate in each group at each visit will be summarized by number and percentage of subjects, and the 95% CI will be calculated. Inter-group differences will be compared using chi- square test or Fisher's exact test.

Refer to the Statistical Analysis Plan (SAP) for details.

8.11.5 Analysis plan

First-stage analysis:

The first analysis will be performed after the 28-day safety data have been reviewed.

Second-stage analysis:

The second analysis will be performed after the 28-day immunogenicity data have been reviewed.

Final analysis:

The final analysis will be performed after the 6-month safety and immunogenicity data have been reviewed.

8.11.6 Analysis software

SAS version 9.4 or above is utilized for analysis in this study.

9 CLINICAL TRIAL MONITORING

9.1 Responsibilities of the Parties

9.1.1 Investigator

The principal investigator is responsible for management and assignment of responsibilities for all personnel participating in the clinical trial.

The investigators shall keep the personal information of subjects confidential. Source records, eCRF, and other documents should only be identified by subject code and the randomization number. The investigators will save the identification list and screening registration forms of the subjects (including the full names, ages, and addresses) in the Investigator Site File. As per the GCP principles, the raw data of each subject is allowed to be monitored, audited, and reviewed.

9.1.2 Clinical research associate

The clinical research associate (CRA) shall perform the on-site follow-up monitoring regularly. During the monitoring, whether the raw data is consistent with the information on the eCRF will be verified, i.e., the accuracy and completion status. If the eCRF is found inconsistent with the raw data, it is necessary to urge the investigators to modify it. The CRA will evaluate the process of informed consent, the transportation and storage status of the investigational vaccine, the trial documents and the progress of the trial. The CRA will check the compliance of the investigators with the protocol (or protocol amendment), observe the procedures of the trial, and discuss the issues with the investigators. There should be monitoring records to document on-site monitoring.

9.2 Personnel Training

The trainees include site operators, laboratory operators, and clinical medical personnel. Training contents include study-related contents, study process, clinical techniques, and emergency handling.

9.2.1 Site operators

All the personnel participating in the clinical trial are investigators and are required to present medical/nursing professional qualification certificates (including medical practitioner certificate, and medical technician work license). Site operators are required to provide CVs (signed and dated) and relevant documents. These materials are filed in the training documents by the responsible person. All those participating in the training are required to register in the training registration form. A brief description of training topics or tasks shall be provided in the training meeting records, including reference from relevant documents, version number, and training date. Training shall be provided as appropriate by the responsible person when new staff are assigned to new posts. The training content shall be relevant to the (new) posts of the staff, and practical training is required.

9.2.2 Laboratory operators

Laboratory operators shall present qualifications above technician. Laboratory operators are allowed to perform laboratory operations only after they have received training in terms of the GCP, trial protocol, and SOPs, passed the examination, and been authorized by the principal investigator.

The contents of evaluation include:

1) quality of work;
2) mastery of new techniques and procedures;
3) observance of relevant technical instructions and procedures;
4) ability of appropriate explanation to results;
5) ability of error avoidance;
6) whether the test results meet the acceptance criteria defined in relevant documents;

Additional training must be performed in case new or modified testing processes or techniques are to be implemented.

9.2.3 Clinical medical personnel

Doctors are required to provide the identification of physician's qualification or medical practitioner certificate; nurses are required to provide the professional certificate of nursing; lab technicians are required to provide the work license.

Emergency doctors and nurses shall possess professional qualities, and techniques including cardiopulmonary resuscitation, shall be currently engaged in emergency work and acquainted with the emergency treatment for common vaccination adverse reactions, especially immediate hypersensitivity reaction, and be acquainted with the process of emergency green channel.

The principal investigator or CRA should provide training for each medical personnel related to each function. Refer to the GCP and "Field Operations Manual".

9.2.4 Clinical research associate (CRA)

According to the "Drug Registration Regulation", the applicant should designate personnel with certain expertise to supervise the implementation of "Good Clinical Practice for Vaccine Trials" (draft) during the clinical trial. The CRA participating in this study shall be acquainted with the following:

1) "Good Clinical Practice" (GCP) and other regulations;
2) "Quality Guidelines for Clinical Trials of Vaccines" (Tentative)
3) Clinical trial protocol;
4) Informed consent form;
5) Storage, transportation, dispensing and use of vaccines;
6) Forms used at the vaccination site and requirements for filling out the forms.

9.3 Test Reagents and Methods (Sample Testing Institution)

9.3.1 Reagent management

1) Standardized test methods shall be used;
2) Quality monitoring shall be performed at irregular intervals;
3) Reagents shall be stored at specified temperatures in places with a temperature monitoring system for storage status monitoring. The cabinets and rooms for reagents stored at room temperature shall be locked;
4) Upon receipt of reagents, the expiry dates shall be recorded in the reagent management log;
5) All reagents stored shall be checked regularly for expiry. Expired reagents shall be disposed of as regulated;

6) When reagents are opened for use, the initials of the user, opening date and expiry date shall be indicated on the bottle.

9.3.2 Test methods

Tests shall be carried out in accordance with various sample testing SOPs.

9.4 Vaccine Management

The investigational vaccines shall be managed by designated personnel. Access to the vaccine is not allowed without permission by the principal investigator. The vaccines should be transported and stored between 2–8 °C. The investigator is responsible for regularly exporting the temperature records of the refrigerator storing the vaccines. The investigator should be notified whenever temperature deviations occur, i.e., a temperature outside the specified range of 2–8 °C.

Forms for vaccine handover, registration, use, and recovery are established, completed as per requirements, and filed in the work records. This is to ensure the quality of investigational vaccines, and prevent non-marketed vaccines from entering the market.

1) Vaccine handover records: CanSino Biologics Inc. will provide the investigational vaccines and the "Vaccine Handover Form". Upon receipt of vaccines, the investigator will verify the quantity of the vaccine, integrity of the packaging, and whether the cold chain system indicator is normal. The "Vaccine Handover Form" shall be signed by both parties.

2) Vaccine registration and use records: The investigator shall establish vaccine registration and use records to record in details the quantity of vaccines inoculated, remaining, or wasted each day.

3) Vaccine return records: The investigator shall return all remaining investigational vaccines and the "Vaccine Handover Form".

4) At the end of the project, vaccines may be destroyed on site or returned to the manufacturer with the concurrence of the manufacturer. The principal investigator is responsible for explaining any discrepancies in vaccine quantity.

9.5 Site and Laboratory Supervision

The site CRA is responsible for supervising the whole process of the clinical trial to ensure that the contents of the trial comply with the requirements of GCP and clinical trial protocol, and the trial is completed within the expected time.

1) To supervise the storage, transportation, dispensing and use of vaccines, as well as destroying of remaining vaccines;

2) To participate in subject enrollment, sample collection, vaccination and side effect observation, laboratory testing, statistical analysis of results, and clinical report preparation;

3) To perform monitoring at the study site regularly and submit a written supervision report of the whole process of the trial to the sponsor and the principal investigator of the clinical trial institution in time (in 3 days);

4) The site CRA is responsible for resolving any protocol deviations during the clinical trial with the investigator, and report major events to the Ethics Committee.

9.6 Quality Control of the Document Materials

9.6.1 Raw data

9.6.1.1 Investigator site document materials

All site document materials are categorized and preserved. Document types are as follows (subject to change per site conditions):

1) Source record;

2) Informed consent form;

3) Sample collection record;

4) Vaccination record;

5) Cold chain record;

6) Vaccine handover, use, dispensing, and recovery record;

9.6.1.2 Laboratory document materials

1) Sample test result and record (test report)

The following documents are included in the lab process from sample receipt to final result report:

a) Transportation: sample transportation form, sample transportation issue log, and sample invalidation record;

b) Sample tracking: internal sample transition form;

c) Sample testing: serum antibody test;

2) Statistical analysis data of sample test results

The raw data of sample test results is entered by a laboratory operator into an Excel file saved in the lab computer. The data entered is reviewed by another laboratory operator. The Excel file will be transferred to the data manager upon completion of data entry review.

9.6.2 Electronic case report form (eCRF)

Two copies of eCRFs will be transcribed for each subject by the investigator. One copy of the eCRF is provided to the study center and the other is retained by the investigator. During the study, only the investigators and authorized staff are permitted to access the eCRFs.

The eCRF is used to record clinical trial data, and is an important part of clinical trials and study reports.

Whether a subject completes the trial or withdraws from the trial, the investigator must sign the eCRF to declare that the data recorded is accurate. For subjects who terminate the trial, the reason for termination shall be recorded in the eCRF.

The eCRF shall reflect the situation of subjects at each stage during the trial. The appropriate code and subject initials, instead of the subject's name, shall be used in the eCRF.

All data in the eCRF is derived from and consistent with the raw data. All data recorded in the eCRF shall be traceable in the raw data.

The investigators and other relevant personnel should provide written documentation for correspondences and meetings related to the study as well as protocol amendments. All mutually agreed documents should be kept in duplicate and stored separately.

9.6.3 Data storage

All clinical trial documents must be retained in accordance with GCP requirements.

9.7 Quality Control of Biological Samples

9.7.1 Quality control of collection and handling of biological specimens

Blood samples that are used for serum antibody tests should be centrifuged and aliquoted within 8 h after collection; serum hemolysis rate ≤ 2% and error rate ≤ 1%. PBMC should be separated within 4 h after collection for ICS assay. Preservation solution should be added to saliva samples immediately after collection.

Collectors should verify the basic information and procedures in the source record and perform local sterilization prior to blood collection. Saliva samples should be handled as per the SOP. After sample collection, subject ID and sample number must be noted in relevant documents and labeled on the blood collection tubes, and the information must be verified. After blood collection and

numbering, collectors should sign the source record. Special circumstances during blood collection should be recorded accurately.

Designated personnel shall inspect the blood collection process, saliva samples, sample quality, as well as the quality of documentation. In the case of wrong numbers, repeated numbers, or invalid samples, the site director should be notified immediately to correct the error.

Samples collected shall be stored properly and transferred to laboratory blood separation personnel in time with handover record. Medical wastes shall be placed as per classification requirements and transferred to relevant responsible persons for disposal.

9.7.2 Quality control of transportation of biological specimens

All samples must be transported to the laboratory and documented as per regulations.

The on-site logistics administrator shall prepare the sample transportation form before sample delivery, which shall contain the quantity of samples, sample box number, and sample number. A written sample transportation form is delivered together with the samples.

Upon receipt of the samples, the laboratory sample receiver shall check the quantity and status of samples, consistency between samples and the sample transportation form, and uniqueness of sample number, and sign on the sample transportation form thereafter.

Temperature monitoring records are required during the transportation of serum and saliva samples.

9.7.3 Quality control of biological sample storage

Temperature of all refrigerators related to the project shall be monitored once each in the morning and evening. In case of temperature deviation, the responsible person is required to record the reason for deviation and actions taken in the temperature monitoring form, including management like cold chain interruption warning.

10 RISK MANAGEMENT PLAN

10.1 Safety Specifications

Safety specifications include important identified risks, important potential risks, and important missing information. Full consideration is required based on the ADRs collected and summarized in prior clinical studies, risks of medical treatment/intervention requirement, reactions of the same category, indications, epidemiology of the targeted population, safety risks observed in non- clinical studies (including toxicology and drug interaction), populations not studied in the clinical trial, etc.

10.2 Pharmacovigilance Plan

SUSARs and potential safety risks of the investigational vaccine are closely monitored and reported by referring to safety-related study data, literatures, or reports on domestic and foreign vaccines of the same kind; in case of major safety risk warning or report, a risk control plan shall be prepared, and necessary actions shall be taken to protect subject safety. Safety monitoring data in the clinical trial shall be regularly summarized and analyzed according to relevant requirements. Safety risk signals of the product shall be focused during the analysis of monitoring data. The difference between the monitoring data and the safety information in the product package insert is further identified based on the analysis of monitoring data. The incidence of new and serious adverse reactions is analyzed for discussion of the necessity of risk management actions and benefit/risk assessment opinions.

10.3 Risk Minimization Actions

Main measures include timely update and revision to inclusion and exclusion criteria of the protocol, notes in the Investigator's Brochure, and the informed consent form, etc. according to the information collected in the long-term observation and follow-up; additional risk minimization actions include risk grading of identified risks and provision of recommendations for actions, enhanced communication with subjects, and provision of corresponding training to personnel participating in the study to spread relevant handling recommendations for risks. In the trial protocol, uniform criteria and methods for safety evaluation are formulated for active monitoring and follow-up of vaccine safety as per corresponding guidelines issued by NMPA.

11 SCHEDULE

The total study duration is planned to be approximately 15 months.

12 ETHICAL APPROVAL

12.1 Review and Approval

The PI is responsible for submitting the clinical trial protocol and all necessary documents to the EC of each center for initial review. Written approval should be provided by the EC to the investigator.

The investigator shall also provide a sample informed consent form to the Ethics Committee for review and approval.

Volunteers should be given sufficient time to consider whether to participate in this trial before signing the informed consent form. The subjects have opportunities to inquire about study details and receive detailed answers. During the trial, the subjects have the right to withdraw from the study.

12.2 Processes to Be Supervised

12.2.1 Informed consent

Whether the methods of recruiting subjects and relevant information provided to the subjects are complete and accessible, and whether the methods of obtaining informed consent are proper shall be supervised. During the whole process of the trial, the Ethics Committee shall supervise the existence of issues harmful to subject in ethical term and whether the subject receives treatment or compensation for damage due to the trial, and assess the extent of risk the subject is exposed to.

12.2.2 Confidentiality

The personal information of subjects shall be kept confidential during the trial, biological sample collection, report, and publication. Only the ID, randomization number, and sample number are labeled on biological samples.

12.2.3 Potential risks and risk minimization

1) Vaccination

Data from prior clinical trials showed that the investigational vaccine is safe, and the adverse reactions are mostly mild and transient. If an adverse reaction is considered related to vaccination (abscess at the inoculation site), timely treatment as per relevant regulations will be provided. If a life-threatening SAE occurs, the subject will be sent to a cooperative hospital for treatment immediately through Green Channel.

2) Sample collection

Under stringent supervision, experienced medical care personnel perform sample collection according to the procedures as stipulated after training, so as to minimize the pain or risk of subjects.

During the whole process of the trial, the Ethics Committee shall supervise the existence of issues compromising subject ethics, whether the subject receives treatment or compensation for damage due to the trial and the actions taken, and assess the extent of risk the subject is exposed to.

13 DATA DISCLOSURE AND PUBLICATION

If the study results are required to be disclosed and/or published after the clinical trial, the positive and negative results will be disclosed and/or published together.

14. REFERENCES

[1] Zhu, Feng-Cai et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. The Lancet, Volume 395, Issue 10240, 1845-1854.

[2] Zhu, Feng-Cai et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. The Lancet [J] 2020; S0140-6736(20)31605-6.

[3] Lu RJ, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet, 2020 https://doi.org/10.1016/S0140-6736(20)30251-8.

[4] Shipo Wu, Jianying Huang, Zhe zhang, et al. Safety, tolerability, and immunogenicity of an aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in adults: preliminary report of an open-label and randomised phase 1 clinical trial. The Lancet Infectious Diseases [J], July 26, 2021, DOI: https://doi.org/10.1016/S1473-3099(21)00396-0.